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CONTENTS

SEC. C.—BOTANICAL SCIENCES

Page

Varietal Differences in Barleys and Malts. VI. Autolytic Proteolytic Activity of Malt and Its Correlations with Wort Nitrogen and Barley Nitrogen Fractions—*C. A. Ayre and J. A. Anderson* - - - - - - - - - - - - 239

Physiological Activity of a Series of Naphthyl Acids—*N. H. Grace* - - - - - - - - - - - - - - - 247

Nitrification Under and After Alfalfa, Brome, Timothy, and Western Rye Grass. II. Soil Microbiological Activity—*J. D. Newton, F. A. Wyatt, V. Ignatieff, and A. S. Ward* - - 256

Researches on Drought Resistance in Spring Wheat. I. A Machine for Measuring the Resistance of Plants to Artificial Drought—*C. B. Kenway and H. B. Peto* - - - 294

SEC. D.—ZOOLOGICAL SCIENCES

An Apparatus for Measuring the "Flash" Thermal Death Point of Microscopic Animal Organisms and Its Use with Ova of *Ascaris lumbricoides*—*W. E. Swales and D. K. Froman* 169

Food of Ducks and Coots at Swan Lake, British Columbia—*J. A. Munro* - - - - - - - - - - - - - - 178

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VARIETAL DIFFERENCES IN BARLEYS AND MALTS

VI. AUTOLYTIC PROTEOLYTIC ACTIVITY OF MALT AND ITS CORRELATIONS WITH WORT NITROGEN AND BARLEY NITROGEN FRACTIONS[1]

BY C. ALAN AYRE[2] AND J. ANSEL ANDERSON[3]

Abstract

The proteolytic activity of 144 samples of malt, representing 12 varieties grown at 12 experimental stations in Canada, was determined by an autolytic method. Certain varieties differed widely in average activity (Olli, 291; O.A.C. 21, 235; and Wisconsin 38, 150 units), those of poor malting quality tending to give low values. The spread between station means was also large (Beaverlodge, 284; and Nappan, 149 units).

The correlations between proteolytic activity, barley nitrogen fractions, total barley nitrogen, and wort nitrogen (data given in an earlier paper), were also studied. *Intra-varietal* partial correlations independent of total nitrogen, between proteolytic activity and nitrogen fractions, were all insignificant. Corresponding *inter-varietal* partial correlations were insignificant for insoluble and alcohol-soluble nitrogen, but highly significant for salt-soluble barley nitrogen and wort nitrogen. A close *inter-varietal* relation was found between proteolytic activity and salt-soluble barley nitrogen, and it was impossible to demonstrate that these two properties influenced wort nitrogen independently.

Varietal differences in the percentage of barley nitrogen appearing in the wort extracted from malts, or differences in some fraction of this wort nitrogen, have been demonstrated by several investigators (3, 4, 12, 13, 15, and others), and it has seemed reasonable to suppose that these differences reflected differences in the amounts of proteolytic enzymes elaborated or liberated during the malting process. In the preceding paper of this series (2), however, it was shown that a fairly high inter-varietal relation ($r = 0.88$) existed between wort nitrogen and the salt-soluble nitrogen of the barley from which the malt is made. It thus appeared that wort nitrogen might be a function of both salt-soluble barley nitrogen and proteolytic activity, and might therefore be a rather poor measure of the latter. Alternatively, it also seemed possible that both salt-soluble barley nitrogen and wort nitrogen might be functions of proteolytic activity, and that the correlation between the two nitrogen fractions might be merely an expression of these relations.

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The work reported in this paper, a study of the autolytic proteolytic activity of 12 barley varieties, besides demonstrating that large varietal differences exist, also provided data bearing on the questions discussed above. Statistical analyses showed that a close inter-varietal relation exists between salt-soluble barley nitrogen and proteolytic activity of malt, but it was not possible to demonstrate that these two properties influenced wort nitrogen independently.

Materials

The malts used in this study are those used in previous studies in this series. They represent 12 varieties of barley grown at 12 widely separated experimental stations in Canada (listed in Table I). The barley varieties and the methods used in growing the samples were described in detail in Part I (1) of this series and the malting methods and commonly measured characteristics of the malts were reported in Part IV (12).

Method

A considerable number of methods for evaluating the proteolytic activity of malts have been developed. In the majority of these an aqueous extract of the malt is made, and its activity is measured by the change in viscosity of a gelatin substrate (6, 9, 10), or by its hydrolytic action on an edestin substrate (7, 9). Laufer (9) reports that no agreement exists between the results of representative gelatin and edestin methods. Idoux (5) used ground barley, in which enzymes were said to have been destroyed by drying at 115° C. for 4 hr., as a substrate. Kolbach and Simon (7) used an autolytic method in which the malt protein acts as substrate. They attribute the greater portion of proteolytic activity to insoluble enzymes and have shown that results obtained from an autolytic digestion differ widely from those obtained by allowing an aqueous extract of the malt to act on edestin. They prefer the autolytic method. Lüers (11) also recommends the use of the natural malt protein substrate.

The method selected for the present investigation involved measuring the amount of non-protein nitrogen produced during the second and third hours of digestion in a malt mash buffered to pH 4.6 and maintained at 45° C.

The method is by no means free from objections. Differences in the protein distribution among samples of the same variety grown at different stations make the interpretation of station differences in autolytic proteolytic activity difficult, if not impossible. On the other hand, since inter-varietal differences in protein distribution are reasonably small, comparisons of autolytic values for samples of different varieties grown at the same station can probably be considered fairly reliable.

Typical curves showing the relations between non-protein nitrogen produced and time of digestion, for samples of two varieties grown at the same station, are shown in Fig. 1. These show that the method is not particularly precise, that the rate of proteolysis is only approximately constant over the period selected for measurement (1 to 3 hr.), and that the rate begins to decrease with longer periods of digestion.

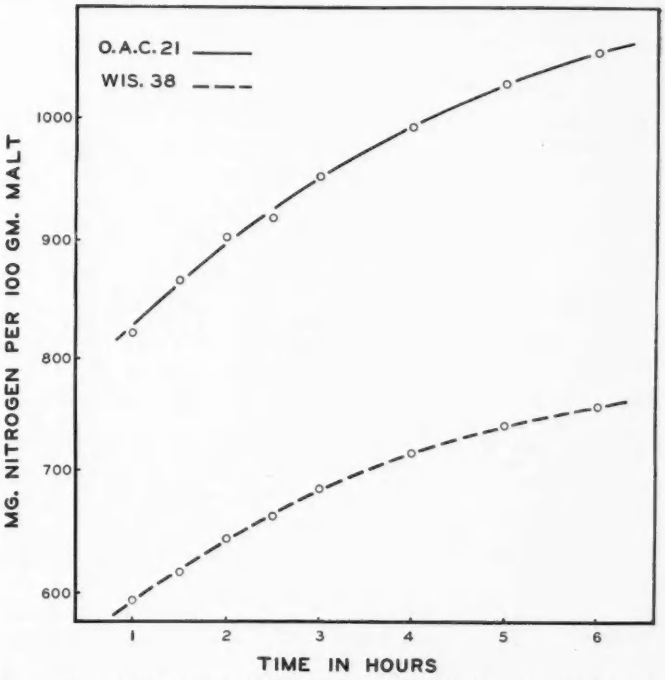

FIG. 1. *Non-protein nitrogen produced by autolytic digestion of malts for successive time intervals.*

Details of Method. A 25-gm. sample of malt was finely ground in a Seck mill. Two 10-gm. aliquots were weighed into 100-ml. glass centrifuge tubes, and 50 ml. of a sodium acetate-acetic acid buffer solution, pH 4.6, were added to each tube with rapid stirring. The tubes were placed in a water bath at 45° C., and the mashes were stirred at 5-min. intervals.

The proteolysis in one aliquot was stopped after 1 hr. by adding 10 ml. of 30% trichloracetic acid solution. After stirring and centrifuging, the supernatent liquid was filtered and the nitrogen (*i.e.*, non-protein nitrogen) in 20 ml. of the filtrate was determined by a Kjeldahl method. This determination replaces the blank determination. It was made after 1 hr. of digestion, rather than earlier, in order to allow the original soluble constituents of the malt sufficient time to dissolve completely.

The proteolysis in the second aliquot was stopped after 3 hr., and the non-protein nitrogen was determined as previously described.

Proteolytic activity is calculated from the difference between the amounts of non-protein nitrogen in the 1- and 3-hr. digestions.

Replication. In order to provide a check on the precision of the determination during the investigation, duplicate determinations were made on

one-third of the samples. These were selected at random, after imposing the limitation that four samples of each of the 12 varieties and four samples from each of the 12 stations should be chosen. The standard deviation of the mean of duplicate determinations, calculated from 48 pairs of values, was ± 13.2 for a mean value of 220 mg. of non-protein nitrogen, per 2 hr., per 100 gm. of malt. Precision did not prove to be a limiting factor in the comparisons of either varietal or station means.

Results and Discussion

Varietal and Station Differences

The results of the investigation are summarized in Table I as varietal means, over all stations, and as station means, over all varieties. Owing to the differential effect of environment on varieties, these did not fall in the same order with respect to proteolytic activity at all stations. For the same reason the stations did not fall in the same order within each variety. An analysis of variance was necessary to determine whether the differences between varietal and station means could be considered significant. The results of the analysis are recorded in Table II and show that both varietal and station differences are highly significant. To facilitate comparisons the necessary differences between means for a 5% level of significance, *i.e.*, for odds of 19 to 1 that a real difference is operating to spread the means, are given in the last line of Table I.

TABLE I

PROTEOLYTIC ACTIVITY OF MALTS: MEANS FOR EACH VARIETY AND EACH STATION

Class	Variety	Proteolytic activity*	Station	Proteolytic activity*
Six-rowed, rough-awned	O.A.C. 21	235	Nappan	149
	Mensury, Ott. 60	230	Fredericton	192
	Olli	291	Ste. Anne de Bellevue	199
	Peatland	220	Ste. Anne de la Pocatiere	218
	Pontiac	214	Lethbridge	195
Six-rowed, smooth-awned	Nobarb	100	Winnipeg	225
	Regal	196	Brandon	213
	Velvet	210	Guelph	219
	Wisconsin 38	150	Ottawa	210
Two-rowed, rough-awned	Charlottetown 80	264	Lacombe	244
	Hannchen	225	Beaverlodge	284
	Victory	196	Gilbert Plains	242
Necessary difference, 5% level		29		29

* *Proteolytic activity is reported as milligrams of non-protein nitrogen produced by 100 gm. of malt during the second and third hours of an autolytic digestion.*

Comparison of the actual differences between varietal means with the necessary difference leaves no room for doubt that proteolytic activity, as measured in the present investigation, is a varietal characteristic. These

results are thus in line with those of Wahl (14) and Koch *et al.* (6) who have previously made small studies of varietal differences in proteolytic activity using other methods of measurement.

TABLE II

ANALYSIS OF VARIANCE FOR PROTEOLYTIC ACTIVITY

Variance due to	Degrees of freedom	Mean square
Varieties	11	18,415.8**
Stations	11	13,103.0**
Remainder	121	1,306.9

NOTE: *In this and later tables,* ** *denotes that the 1% level, and* * *that the 5% level of significance is attained.*

There is no evidence that different classes of barley are characterized by differences in proteolytic activity. The rough-awned, six-rowed variety, Olli, gives the highest values and is followed by the two-rowed variety, Charlottetown 80. Eight of the other varieties form an intermediate group covering a range of 40 units. Two of the smooth-awned, six-rowed varieties, Nobarb and Wisconsin 38, give very low values.

In Canada it is generally agreed that among the six-rowed, rough-awned varieties, O.A.C. 21, Mensury and Olli are best for domestic malting purposes, and that among the smooth-awned group, Velvet is much less unsatisfactory than the others. The data in Table I thus indicate that fairly high proteolytic activity is an attribute of varieties of good malting quality.

A study of the station means also suggests that proteolytic activity is affected by environment. The stations are listed in order of increasing nitrogen content of their samples (*cf.* (1, Table I)). Inspection will thus show an apparent increase in proteolytic activity with increasing total nitrogen. It should be borne in mind, however, that increasing amounts of nitrogen substrate together with regular changes in the distribution of the nitrogen among various protein fractions (*cf.* (1, Table II)), may affect the apparent proteolytic activity. It would thus be unwise to attempt to draw definite conclusions about the effect of environment on proteolytic activity from the data presented in Table I.

The authors have considered the possibilities of correcting for the effect of differences in the nitrogen contents of samples from different stations, either by reporting proteolytic activity in terms of the percentage of nitrogen hydrolyzed, or by making adjustments by means of the analysis of variance and covariance method (*cf.* (1, p. 388)). It appears, however, that to make corrections by either method, certain unjustifiable assumptions must be made. In these circumstances it seems preferable to let further research elucidate the issues involved.

Relations between Proteolytic Activity and Nitrogen Fractions

Correlation coefficients showing the relations between proteolytic activity on the one hand, and total barley nitrogen, barley nitrogen fractions, and wort nitrogen, on the other, are given in Table III. The independent variables are listed in the first column, and the relation between proteolytic activity and each of these is represented by a row of four correlation coefficients, namely, the simple and partial correlation coefficients for varieties and stations.

TABLE III

RELATIONS AMONG PROTEOLYTIC ACTIVITY (p) NITROGEN FRACTIONS (x) AND TOTAL NITROGEN (n)

x = independent variables listed below	Correlation coefficients			
	Varieties		Stations	
	Simple r_{px}	Partial $r_{px.n}$	Simple r_{px}	Partial $r_{px.n}$
Total nitrogen	.070	—	.854**	—
Insoluble protein nitrogen	−.474	−.539	.792**	.006
Alcohol-soluble protein nitrogen	−.098	−.265	.888**	.297
Total salt-soluble nitrogen	.871**	.881**	.578*	−.545
Salt-soluble protein nitrogen	.870**	.871**	.545	−.235
Non-protein nitrogen	.741**	.780**	.683*	−.064
Wort nitrogen	.874**	.877**	.607*	−.136
Residual degrees of freedom	10	9	10	9

Comparison of the data given in the first two columns of Table III shows that inter-varietal differences in total nitrogen content have very little effect on the inter-varietal relations between proteolytic activity and nitrogen fractions. The correlation coefficients have essentially the same values irrespective of whether an adjustment is or is not made for varietal differences in total nitrogen content. The statistics show that no inter-varietal correlation exists between proteolytic activity and total nitrogen, insoluble protein nitrogen or alcohol-soluble nitrogen. On the other hand, it is shown that proteolytic activity is directly correlated with the more soluble barley nitrogen fractions and with wort nitrogen: varieties that tend to be high in proteolytic activity also tend to be high in content of salt-soluble barley nitrogen, and tend to produce malts yielding worts of higher nitrogen content.

The correlation coefficients for stations present a different picture. The fact that the simple correlation coefficients are significant, whereas the partial correlation coefficients are not, shows that the former merely reflect the correlations between proteolytic activity and total nitrogen, and between the independent variables and total nitrogen (*cf.* (1, Table VI)). When the complicating effect of station differences in total nitrogen is removed by calculating partial coefficients, it becomes apparent that the experimental data fail to demonstrate that any intra-varietal relation exists between proteolytic activity and the various nitrogen fractions. The data show only

that within varieties, environmental conditions that tend to increase total nitrogen content also tend to increase proteolytic activity.

Relations between Wort Nitrogen, Proteolytic Activity and Total Salt-soluble Barley Nitrogen

In Part V of this series (*cf.* (2, Table I)), it was shown that a highly significant intervarietal correlation ($r = 0.887$) exists between wort nitrogen and total salt-soluble barley nitrogen. Since inter-varietal correlations of the same magnitude exist between both these nitrogen fractions and proteolytic activity (see Table III), further investigation of the inter-relations among these three properties seemed desirable. The correlation coefficients given in Table IV help clarify these relations. The simple correlation between

TABLE IV

RELATIONS BETWEEN WORT NITROGEN, PROTEOLYTIC ACTIVITY, AND TOTAL SALT-SOLUBLE BARLEY NITROGEN

Correlation between	Correlation coefficient
Wort nitrogen and salt-soluble barley nitrogen	
Simple	.887**
Partial, independent of proteolytic activity	.528
Wort nitrogen and proteolytic activity	
Simple	.874**
Partial, independent of salt-soluble barley nitrogen	.448

wort nitrogen and salt-soluble nitrogen is highly significant, but the partial correlation, independent of proteolytic activity, is insignificant. The relation between wort nitrogen and proteolytic activity is very similar: the simple correlation is significant, but the partial correlation independent of salt-soluble nitrogen is insignificant. These relations appear to show that salt-soluble nitrogen and proteolytic activity are directly related; because, when the covariance between salt-soluble nitrogen and the other two factors, *i.e.*, proteolytic activity and wort nitrogen, is removed, most of the covariance between the two latter factors is removed also. It should also be noted that this relation makes it impossible to obtain a significantly higher correlation between wort nitrogen and salt-soluble nitrogen by introducing proteolytic activity as a second independent variable. The multiple correlation coefficient proved to be 0.891, which is not significantly higher than the simple correlation, 0.887.

In these circumstances it is impossible to differentiate between the parts played by the proteases and the salt-soluble nitrogen fraction of barley in producing wort nitrogen. All that can be said is that varieties that tend to contain larger amounts of salt-soluble nitrogen also tend to produce malts of higher proteolytic activity which yield worts of higher nitrogen content.

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PHYSIOLOGICAL ACTIVITY OF A SERIES OF NAPHTHYL ACIDS[1]

By N. H. Grace[2]

Abstract

An homologous series of ω-naphthyl, aliphatic acids from the acetic to the hexoic has been presented to the author by Dr. R. H. Manske, and the physiological activity of these has been determined by the rooting response of plant cuttings treated with solutions of each. Statistically significant positive effects have been noted on the number of cuttings that rooted, the number and length of roots per rooted cutting, and the mean root length. The results with several plant species indicate that activity exists up to and including naphthyl hexoic acid, the highest member of the series tested. A noteworthy feature of the results is the activity of the acids with an even number of carbon atoms in the side chain; those with an odd number have activity of a lower order.

An homologous series of naphthyl acids has been prepared and reported by Dr. R. H. F. Manske (3). It has been observed that 1-naphthylacetic acid possesses properties similar to those of indolyl-3-acetic acid in affecting certain plant responses, and that a mixture of 1- and 2-γ-naphthylbutric acid also has a measure of activity (2, 5). It is therefore of both theoretical and practical interest to determine the activity of other members of the series. Accordingly, the physiological activity of the acids from 1-naphthylacetic to ε-(1-naphthyl)-hexoic has been determined by the rooting response of treated cuttings. In addition, the series of treatments included naphthylene-1, 5-diacetic acid, indolyl-3-acetic acid, and a control, making eight treatments.

Experimental

The activity of the various acids was determined by the rooting responses of both summer collections of greenwood and herbaceous material and winter collections of dormant current year's growth.* Cuttings were treated by immersing the basal end in solution for a period of 22 hr. for summer, and 24 hr. for dormant, material. Controls were treated in a 100 p.p.m. solution of potassium acid phosphate; solutions of the acids were prepared in phosphate of this concentration, as solubility in water is extremely low for some of the members of the series.

In the earlier experiments the acids were used at five concentrations, 100, 50, 25, 10, and 5 p.p.m. of solution. There were three replicates, with seven cuttings in each group, but in one experiment in which the material was limited, five cuttings were used for each of the eight treatments. The total number of cuttings was 840. In some of the earlier experiments individual treatments were carried out in separate beakers, enabling determination of

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**The prepared cuttings were supplied by the Federal District Commission through the kindness of Mr. E. I. Wood.*

the change in weight accompanying treatment. Subsequently, all the replicates of one treatment and concentration were treated together. In a later experiment, with dormant cuttings of *Lonicera tartarica*, there were ten cuttings to the group with only three concentrations, 100, 50, and 10 p.p.m. In this experiment there were five replicates and 1200 cuttings. The amount of solution used in treatment varied from 50 cc. for groups of seven small cuttings to 150 cc. when five replicates of ten large cuttings were treated in the same beaker, but was constant throughout any given experiment.

The design of the experiment provided for analyses of variance of the observations. Each replicate, or complete block, contained five incomplete blocks, each comprising the eight treatments at one concentration in random order. Treatments were further randomized within incomplete blocks. All the summer cuttings were planted in brown sand in cotton-covered propagation frames in a garden. Dormant winter cuttings were placed in brown sand in frames equipped with bottom heating electrical cables and situated in a greenhouse. The room temperature approximated 65° F., while the sand temperature was maintained at 72° F. When the cuttings were removed, record was made of the number rooted, the number and length of roots per rooted cutting and, in some experiments, the mean root length.

Results

Each experiment was taken from the propagation frame when preliminary observation indicated that substantial rooting had occurred. The results described in the following paragraphs are arranged under the headings of the different plants used.

Viburnum Opulus L.

Cuttings of this plant were treated August 5 and removed from the propagation frame September 10, 1938. Measurements were made immediately on the rooted cuttings.

The data in Table I are for the change in weight of groups of seven cuttings that had received a 22-hr. solution treatment, for the number of cuttings rooted, and for the mean number of roots per rooted cutting. Statistically significant effects on the weight of cuttings occurred following treatment with each of the six naphthyl acids and with indolylacetic acid. There are significant differences within concentrations of each of the naphthyl acids. The butyric and valeric acids cause loss in weight, while naphthyl hexoic shows both marked gain and loss in weight over the concentration range tested. This fact is of interest, as the tendency to form colloidal solutions becomes marked with naphthyl butyric acid and increases with lengthening side chain. Increased rooting follows weight increase with indolylacetic acid at 10 p.p.m. Weight increase of cuttings on treatment with the naphthyl acids either reduces or does not affect the number of cuttings rooted. The hexoic acid at 25 p.p.m. is an exception.

There are significant effects on rooting from each of the chemical treatments at one or more of the concentrations used, with the valeric acid the sole

TABLE I

RESPONSES OF *Viburnum Opulus* CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of three groups of seven cuttings

Concentration, p.p.m.	Control	Indolylacetic acid	Acids of naphthyl series						Necessary difference, 5% level
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic	
Change in weight on treatment, gm.									
100	0.51	0.47	1.50*	1.10*	0.13	−1.13*	0.47	0.33	0.69 between chemicals at one concentration, 0.53 between control and chemicals, and 0.31 between means of chemicals
50	0.51	0.47	0.60	0.87	−0.57*	−0.63*	1.47*	1.03	
25	0.51	1.00	0.10	0.87	−0.40*	−0.23*	1.27*	1.43*	
10	0.51	1.10*	0.63	0.63	−0.47*	−0.03*	0.70	0.80	
5	0.51	0.73	0.67	0.37	−0.17*	0.30	−0.43*	0.60	
Mean	0.51	0.80	0.70	0.77	−0.29*	−0.35*	0.69	0.84*	
Number of cuttings rooted†									
100	2.6	2.7	1.0*	4.0	2.3	2.7	5.0*	4.7*	
50	2.6	1.7	1.7	3.0	5.3*	4.0	2.7	2.3	
25	2.6	3.0	1.7	5.3*	3.3	2.7	5.0*	3.3	
10	2.6	5.0*	5.3*	4.3	4.3	3.7	5.0*	5.3*	
5	2.6	4.3	4.7*	3.7	4.7*	4.7	4.7*	1.0*	
Mean	2.6	3.3	2.9	4.1*	4.0*	3.5	4.5*	3.3	
Number of roots per rooted cutting									
Mean	5.1	9.4*	8.8*	5.2	7.1	4.8	6.1	5.8	2.14

**Values significantly different from the control.*

†Data transformed to $\sqrt{x + \frac{1}{2}}$ *basis (1); necessary differences cannot be given on the untransformed means given above.*

exception. The mean number of rooted cuttings over all concentrations shows naphthyl propionic, butyric, and hexoic acids to have an effect. The relative effects of the chemicals on rooting is observed most clearly from the body of the table, since there is a significant interaction between chemical treatment and concentration. It may be noted that the mean over all concentrations is affected by damage from overdosage, as shown by the 100 p.p.m. treatment with naphthyl acetic acid. Perhaps the most interesting feature of the data is the concentration range over which rooting is stimulated. Naphthyl acetic is damaging at the 100 p.p.m. level and shows a stimulating effect at the 10 and 5 p.p.m. concentrations; naphthyl hexoic is stimulating over the range from 100 p.p.m. down to 5 p.p.m. The results suggest that either the butyric or hexoic acids could be used over a wider range than the acetic, avoiding some of the danger from overdosage associated with the use of solutions of naphthyl acetic acid on cuttings. It must be pointed out that the chemicals are compared on the basis of absolute weight; but the use of equivalent weights would not materially alter the general conclusion.

Analysis of variance of the observations on the number of roots per rooted cutting indicated that the only significant effect was that of chemicals over all concentrations, this being significant to the 1% level. Significant increase in the number of roots follows treatment with both indolyl and naphthyl acetic acids. The number obtained with the butyric acid is not significantly below that with naphthyl acetic and is greater than that with valeric acid. It is apparent that the acids with an even number of carbon atoms in the side chain produce more roots on those cuttings that root than do the acids with an odd number.

Analysis of the observations on the root length per rooted cutting indicated that the data were not significant.

Coleus Blumei Benth.

Cuttings of this plant were treated on August 24 and removed from the propagation frame for measurement on September 23, 1938. The data on the number of rooted cuttings were not analyzed statistically, as this herbaceous plant roots readily and only five cuttings were used to a group. In consequence, the data presented deal with the responses of those cuttings that actually rooted.

In Table II are given data for the number and lengths of roots per rooted *Coleus Blumei* cutting. Considering means over all concentrations it is apparent that naphthyl acetic, propionic, and hexoic acids have significantly increased the number of roots. Each of the acids, excepting the hexoic and diacetic, increases the number of roots at one or more concentrations, the most pronounced effect being that of naphthyl acetic acid, which is effective at all concentrations but 5 p.p.m. and has a much greater effect than indolylacetic acid. There is an apparent falling-off in effect with those acids having four and five carbon atoms in the side chain, activity becoming more pronounced again at the member with six in the chain.

TABLE II

RESPONSES OF *Coleus Blumei* CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of three groups of five cuttings

Concentration, p.p.m.	Control	Indolylacetic acid	Acids of naphthyl series						Necessary difference, 5% level,
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic	
Number of roots per rooted cutting									
100	5.9	12.0*	23.9*	9.0	8.1	6.5	11.4*	7.3	3.6 between mean of control and chemicals, 2.1 between means
50	5.9	9.6*	15.7*	10.2*	5.9	7.5	8.4	5.7	
25	5.9	4.7	10.9*	7.0	4.8	4.2	7.5	4.3	
10	5.9	4.7	12.4*	10.0*	4.2	9.5*	3.7	4.9	
5	5.9	6.2	8.6	9.4	6.7	7.9	8.9	4.9	
Mean	5.9	7.5	14.9*	9.1*	6.0	7.1	8.0*	5.4	
Root length per rooted cutting, mm.									
Mean	154	153	263*	279*	150	207	234	168	83
Mean root length, mm.									
Mean	24	21	19*	29*	24	28	28	29*	4.7

**Values significantly different from the control.*

The only data that are significant for the root length per rooted cutting are the means over all concentrations of the chemicals. These means are significant to the 1% level. Significance may be attributed to the increase in total length of roots resulting from treatment with naphthyl acetic and propionic acids. The effect appears to fall with naphthyl butyric acid, rising with the hexoic, which, while not significantly above the control, gives a greater length of root than the butyric. It is of interest to note that indolylacetic acid failed to show significant effects.

The only significant feature of the data for mean root length is the mean over all concentrations of the chemicals. These are significant to the 0.1% level. It is apparent that treatment with naphthyl acetic acid reduces the length of the individual root; the reduction effected by indolylacetic acid fails to reach significance. The remaining naphthyl acids all increase the mean root length. The increase attains significance with the propionic and diacetic acids. This increase of mean root length is interesting, as solution treatment with growth-promoting substances usually shortens the length of the individual root.

Lonicera tartarica L.

Dormant stem cuttings of *Lonicera tartarica* were treated with solutions of the chemicals on October 18, 1938, and removed for observation 48 days later. The cuttings were approximately 10 in. in length, and were collected before the canes had been subjected to any appreciable frost.

Data are given in Table III for the number of cuttings rooted, the number of roots per rooted cutting, the root length per rooted cutting, and the mean root length. Most of the data attain the 0.1% level of significance. The effect of concentrations on the mean root length is the only one in which merely the 5% level is reached.

All the chemical treatments significantly increase the number of cuttings rooted. Concentrations also are significant, the 100 and 50 p.p.m. levels both giving better rooting than the 10 p.p.m., but not differing between themselves. Since there is no significant interaction between chemicals and concentrations, mean values for rooting over the three concentrations are given. Naphthyl acetic and butyric acids are significantly more effective than either the propionic, valeric, or diacetic acids; the hexoic, however, is not significantly less effective than the acetic or butyric. Naphthyl butyric acid causes significantly better rooting than all other treatments excepting the acetic and hexoic members of the series. Chemicals and concentrations are both very highly significant in their effect on the number of roots per rooted cutting, and the interaction between them is also very highly significant, passing the 0.1% level. The 10 p.p.m. concentration is significantly less effective than the other two levels, which do not differ between themselves. Indolylacetic acid and those naphthyl acids with an even number of carbon atoms in the side chain are significantly more active than the others. Naphthyl valeric acid shows some activity, but of a lower order. The effect of indolylacetic acid, and naphthyl acetic, butyric, and hexoic acids increases in a

TABLE III

RESPONSES OF *Lonicera tartarica* CUTTINGS TREATED IN SOLUTIONS OF CHEMICALS

Data are means of five groups of ten cuttings

Concentration, p.p.m.	Control	Indolylacetic acid	Acids of naphthyl series						Mean value for concentration	Necessary difference, 5% level
			Acetic	Propionic	Butyric	Valeric	Hexoic	Diacetic		
Number of cuttings rooted, transformed data (1)										
Mean	2.50	2.94*	3.10*	2.86*	3.14*	2.88*	2.95*	2.73*	—	0.20
Number of cuttings rooted of 10 planted										
Mean	5.9	8.2	9.1	7.8	9.4	7.9	8.3	7.0	—	
Number of roots per rooted cutting										
100	2.0	8.3*	9.7*	3.1	9.6*	4.3*	5.9*	2.8	5.69	Interaction 1.60, means 0.92, concentrations 0.59
50	2.1	6.1*	9.4*	3.0	8.6*	3.0	6.4*	2.5	5.12	
10	2.8	2.8	5.5*	3.1	5.5*	2.8	3.5	2.4	3.55	
Mean	2.3	5.8*	8.2*	3.0	7.9*	3.4*	5.2*	2.6	—	
Root length per rooted cutting, mm.										
100	128	421*	430*	199	437*	251*	373*	170	301	Interaction 108, means 62, concentrations 22
50	144	353*	443*	191	472*	167	444*	181	299	
10	192	173	333*	214	372*	164	232	166	231	
Mean	155	316*	402*	201	427*	194	350*	172	—	
Mean root length, mm.										
Mean	70	57*	51*	68	56*	60*	66	67	—	9.3

**Values significantly different from the control.*

pronounced manner with concentration. The other acids fail to show this response.

Chemicals, concentrations, and the interaction between them show highly significant effects on the root length per rooted cutting. The 10 p.p.m. concentration is again significantly below that for the two higher concentrations, which do not differ between themselves. Indolylacetic acid and the naphthyl acids with an even number of carbon atoms in the side chain are significantly more effective than all the other treatments. Naphthyl butyric acid is the most active chemical, but is not significantly better than naphthyl acetic. Total root length increases with rising concentration of indolylacetic and naphthyl acetic, butyric, and hexoic acids. However, this interaction between chemical and concentration is not shown by the other members of the series.

The mean root length is reduced by all chemicals excepting naphthyl propionic, diacetic, and hexoic acids. This time the average of the highest concentration, 100 p.p.m., differs significantly from that of the other two, which do not differ between themselves. There is no interaction between chemicals and concentrations. It is apparent that solution treatment with physiologically active chemicals tends to reduce the length of the individual root. It is interesting to note that the hexoic acid, highly active in other respects, fails to show this effect. This may be due to marked change in transport as the length of the side chain increases. Since damage is frequently indicated by the production of masses of short roots by the cutting, it is possible that naphthyl hexoic acid might be used with somewhat less danger of damage from overdosage.

There were significant differences between replicates for both the root length per rooted cutting and the mean root length. Block differences were not significant for the number of cuttings rooted or the number of roots per rooted cutting. It frequently has been observed that root length measurements are subject to fairly marked block differences.

Discussion

The six acids tested all have a measure of activity similar to that of indolylacetic acid, a recognized plant-growth-stimulating chemical. The most precise results were obtained with dormant stem cuttings of *Lonicera tartarica*. This fact is due, in part, to the increased replications used in this particular experiment. It is impossible to make any close comparison of the results obtained with the three different plants; however, significant differences in activity were brought out by each.

It may be pointed out that physiological activity of the chemicals has been demonstrated by two distinct types of observation. The first deals with the initiation of roots, a fact of prime importance in the practical application of chemicals for this purpose. The second includes counts of the number and lengths of root produced and deals only with the responses of those cuttings that actually did produce roots. This type of observation affords definite information, even if an easily rooted plant is employed.

There is, apparently, some decrease in activity as the length of the side chain increases. Responses due to the hexoic acid are usually less marked than those obtained with the butyric or acetic acid. However, the main feature of the results is the greater activity of the acids with an even number of carbon atoms in the side chain. This fact is of particular interest in view of the more frequent occurrence in nature of fatty acids with an even number of carbon atoms. The alternating effect with increasing length of side chain has been mentioned with reference to the indolyl series as determined by the *Avena* test (4).

It is interesting to note that naphthyl hexoic acid has pronounced physiological activity with, apparently, less tendency to cause damage or shortening of the roots than occurs with indolylacetic or naphthyl acetic acids. In consequence, higher members of the naphthyl series having an even number of carbon atoms in the chain may be of value in the treatment of cuttings, particularly with plants in which susceptibility to damage is a serious hazard.

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

II. SOIL MICROBIOLOGICAL ACTIVITY[1]

By J. D. Newton[2], F. A. Wyatt[3], V. Ignatieff[4], and A. S. Ward[5]

Abstract

Soil microbiological activity was measured for eight seasons, 1927 to 1934, in order to study some underlying causes of the comparative effects of alfalfa, brome, timothy, and western rye grass on the yield and nitrogen content of succeeding wheat crops.

When previously fallowed soil was seeded to alfalfa and grasses, the moisture and nitrate content of the soil were reduced, and generally remained at a relatively low level until the sods were plowed up. In the drier seasons the nitrates were reduced to a very low level or disappeared entirely in the grass and alfalfa plots. The nitrate content of the alfalfa plot soils was generally greater than that of the grass plots, and the brome grass plots were generally lower in nitrates than the timothy and western rye grass plots. The wheat plot soils generally contained more nitrate than the grass and alfalfa plots, especially in the drier seasons. When the sods were plowed up, nitrates accumulated in the alfalfa plots to a greater extent than in the grass plots and to a lesser extent generally in the brome plots than in the timothy and western rye plots. The greater nitrate content of the soil under wheat following alfalfa was observed for a period of three or four years in separate sets of plots plowed up two years apart. The nitrate level of the soil under wheat had a tendency to drop in mid-summer, often reaching its lowest point in July. The fallow plot soils were always higher in moisture than any of the cropped plots at the end of each season, and higher in nitrates in the latter half of each season.

The concentration of water-soluble phosphorus was greatest in the surface soil and seemed to be slightly higher under alfalfa and grasses than under wheat, but the total concentration was small and there was no very definite seasonal trend.

The numbers of fungi and bacteria, as determined by the plate count method for five seasons, 1929 to 1933, did not fluctuate very much in certain plots and seasons, but fluctuated greatly in others. The greatest fluctuations in fungal counts were observed under the first crop of wheat following brome grass, and in bacterial counts also under the first crop of wheat following sods, in the relatively moist season of 1931. Plate counts of actinomycetes did not fluctuate very greatly during the one season in which they were determined. The numbers of fungi were generally higher in the alfalfa plots than in the grass plots, but the differences between the grasses were apparently insignificant. Under the first crop of wheat following sods, large Mucor colonies predominated in the alfalfa plot soil plates and the counts were relatively low. Brome grass plot soils gave by far the highest counts of fungi, which consisted mainly of small Penicillium colonies, under the first crop of wheat following sods in 1931. The differences between numbers of bacteria in the alfalfa and grass plots were not very significant. The moisture content of the surface soil fluctuated greatly during most of the seasons. There was evidence of correlation between fluctuations in bacterial numbers and moisture, especially in certain seasons, in all the cropped

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soils. There was less evidence of such correlation in the case of fungi, except under the first crop of wheat following brome grass in 1931. Fallow soil, though normally higher in moisture content in the latter part of each season, did not differ significantly from the grass-cropped soils in counts of fungi and bacteria. Although surface samples usually gave the highest counts, the deeper soil samples (to a depth of three feet) gave fairly high counts of both fungi and bacteria. During the season of 1930, amoebae were determined by the dilution plate count method; more than 1,000 and less than 10,000 per gram were nearly always found in both cropped and fallow soils.

The total nitrogen content of the plot soils showed considerable variation (owing to random sampling) from year to year, but no definite trend downwards or upwards during this period of seven years. The surface soil in every case contained most nitrogen and the subsoil least.

Introduction

This paper deals with soil microbiological aspects of the crop sequence studies at Edmonton described in Part I (19), in which are compared the effects of three grasses and a legume on the yield and nitrogen content of succeeding wheat crops. Soil microbiological activity was measured in order to study some of the underlying causes of the effects produced.

A block of land, divided into three sub-blocks, was seeded without a nurse crop, to alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Levss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), in 1927. These crops were sown in quadruplicate plots, according to the Latin square system, in each of the three sub-blocks. The crop sequence history of the three sub-blocks is shown in the following tabular summary taken from Part I:

	I	II	III
1926	Fallow	Fallow	Fallow
1927	Seeded	Seeded	Seeded
1928	Hay	Hay	Hay
1929	Wheat	Hay	Hay
1930	Wheat	Hay	Hay
1931	Wheat	Wheat	Hay
1932	Wheat	Wheat	Hay
1933	Wheat	Wheat	Wheat
1934	Wheat	Wheat	Wheat

The soil in these plots is a fairly uniform, deep, black loam, rich in organic matter, underlaid by a clay subsoil, and almost neutral in reaction; this soil has been described in earlier publications (34).

Soil microbiological activity, as represented by nitrification, was measured in all plots for the eight seasons 1927 to 1934, except that in 1933 this measurement was confined to Sub-block III. In 1931 the samples were analyzed for water-soluble phosphorus.

Measurements of soil microbiological activity as represented by plate counts of fungi and bacteria (including actinomycetes) were started in 1929 and continued for a period of five seasons. Plate counts of actinomycetes

alone were made in 1929, and in 1930 the numbers of protozoa (amoebae) were determined by the dilution method. A supplementary experiment was carried out in 1932 to obtain a better idea of the distribution of bacteria and fungi in the deeper soil layers.

Supplementary determinations of nitrification and microbial numbers in fallow plots in the same field were made for several years. These fallow plots were outside the experimental block of plots, and were therefore not strictly comparable with the other plots. Nevertheless, it was felt that it would be interesting to make a general comparison of the cropped plots with the very different conditions of the fallow plots.

Total nitrogen determinations were made once a year, on samples of surface, subsurface and subsoil taken for nitrate determinations, to see if any definite trend in total nitrogen content could be measured in the relatively short period of years during which this experiment was carried on.

Methods

Soil Samples

Soil samples for the nitrate determinations were obtained in the following manner: Three or four borings to the depth of $6\frac{2}{3}$ in. were taken from each plot for the surface samples, two borings of $6\frac{2}{3}$ to 20 in. for the subsurface samples, and one or two borings of 20 to 40 in. for the subsoil samples. Composite samples were then made up by thoroughly mixing together the samples from the quadruplicate plots. Thus each surface composite sample was made up of 12 to 16 borings, each subsurface composite of 8 borings, and each subsoil composite of 4 to 8 borings.

The plots were sampled monthly for nitrate determinations. The first samples were taken in May and the last in September. The moisture content of each composite sample of soil was also determined.

Soil samples for microbial counts were taken to a depth of $6\frac{2}{3}$ in., as a rule. Three or four borings were taken from each of the quadruplicate plots, and thus each composite sample was made up of 12 to 16 borings. The samples were placed in clean glass jars and brought to the laboratory, where each composite sample was thoroughly mixed, diluted, and plated as soon as possible. The moisture content of each composite sample of soil was determined, also. Each sampling was done at approximately the same time of the day. The plots were sampled twice a month usually from early May to the middle of September, or about ten times during the season.

Nitrate

The nitrate determinations were carried out by the phenoldisulphonic acid method as modified by Harper (9). The composite soil samples obtained in the field were dried immediately at a temperature of 60 to 70° C. to stop bacterial activity. Higher temperatures were avoided to prevent losses of nitrate by volatilization. The dry samples were ground coarsely and placed in sealers for analysis.

Water-soluble Phosphorus

The Deniges method as modified by Parker and Fudge (20) was used for the determination of water-soluble phosphorus. By this procedure very small quantities of phosphate can be measured with accuracy. A weakness of the method is that it calls for great precision in measuring out the reagents, which causes delay, whereas rapidity is essential in carrying out the determination, because the blue colour developed by reduction of the complex phosphomolybdic acid fades rapidly on exposure to air. A 50-cc. sample of clear solution obtained by filtering the water extract (5 water to 1 soil) through a Buchner funnel was evaporated to dryness and ignited. The residue was taken up with dilute hydrochloric acid and the phosphate thus brought into solution was determined.

Total Nitrogen

The total nitrogen was determined by the ordinary Kjeldahl-Gunning-Hibbard method.

Numbers of Micro-organisms

The nutrient agar cultural plate count method was used for determining numbers of bacteria and fungi. The soil samples were brought into the laboratory in glass sealers and plated with the least possible delay.

The medium used for bacterial counts (including actinomycetes) was a sodium caseinate or nutrose agar medium (8). This medium is easily prepared and gives a reaction of approximately pH 6.8 without special adjustment.

A peptone-glucose acid agar medium recommended by Waksman (8) was used for fungi counts. The reaction was adjusted to pH 3.8 to 4.0 with sulphuric acid. A possible disadvantage of this medium is that it promotes the rapid growth of the proteolytic fungus, Mucor, which in certain cases prevented the appearance of slower developing fungi, such as Penicillium.

For actinomycetes counts, Waksman's nitrate-sucrose agar medium was used (8). This medium has a reaction of approximately pH 7.0.

The amoebae counts were made by the dilution method described by Cutler (4). Sterilized and hardened nutrient agar plates were inoculated with various dilutions, kept moist with sterile water, and examined microscopically after two and four weeks of incubation for presence of amoebae.

A dilution of 1 : 100,000 was generally used for bacterial counts, and 1 : 1000 for fungal counts. The plates were incubated for six days at room temperature for bacterial counts, as a rule, seven or eight days for actinomycetes counts, and two to five days at room temperature for fungal counts.

Results

Nitrification

In Tables I to XVI and in Fig. 1 the weighted average percentage moisture content of the surface, subsurface, and subsoil taken together is shown, and the total nitrate nitrogen content of the soil to the same total depth (40 in.).

TABLE I

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1927

Sub-block	Crop	June 15	July 15	Aug. 13	Sept. 11	Seasonal average
I	Alfalfa	29.6	31.5	24.4	26.4	28.0
	Brome	30.3	30.1	22.6	24.8	27.0
	Timothy	30.2	31.0	24.1	25.4	27.7
	Western rye	29.9	32.3	24.7	26.1	28.2
II	Alfalfa	29.7	33.5	26.7	28.6	29.6
	Brome	30.3	33.2	25.4	27.8	29.2
	Timothy	31.7	29.9	23.5	24.2	27.3
	Western rye	30.5	31.5	24.6	24.8	27.8
III	Alfalfa	31.1	33.5	26.5	27.0	29.5
	Brome	30.6	32.0	26.2	25.6	28.6
	Timothy	30.8	33.2	25.0	25.6	28.6
	Western rye	31.3	30.3	25.9	30.4	29.5

TABLE II

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1927

Sub-block	Crop	June 15	July 15	Aug. 13	Sept. 11	Seasonal average
I	Alfalfa	228.2	193.6	171.0	106.6	174.8
	Brome	180.2	198.2	118.4	102.8	149.9
	Timothy	234.2	209.2	139.0	125.9	177.0
	Western rye	191.6	202.0	133.6	94.2	155.3
II	Alfalfa	235.0	227.8	212.0	150.4	206.3
	Brome	234.4	201.2	175.8	127.0	184.6
	Timothy	239.6	189.2	138.2	108.8	168.9
	Western rye	290.2	202.6	168.2	142.2	200.8
III	Alfalfa	274.4	266.4	243.6	164.8	237.8
	Brome	321.0	231.6	185.2	128.8	216.6
	Timothy	278.0	233.3	194.8	166.0	218.0
	Western rye	319.8	235.8	210.8	137.0	225.8

TABLE III

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1928

Sub-block	Crop	May 17	June 15	July 10	Aug. 16	Sept. 19	Seasonal average
I	Alfalfa[1]	22.7	21.4	25.5	21.4	22.4	22.7
	Brome[1]	20.7	19.9	–	22.8	22.0	21.3
	Timothy[1]	21.8	20.7	25.2	22.1	22.3	22.4
	Western rye[1]	21.5	20.0	24.3	23.4	22.8	22.4
II	Alfalfa	24.8	22.0	25.7	20.2	20.6	22.7
	Brome	22.2	20.7	–	19.3	19.7	20.5
	Timothy	20.6	19.5	23.7	20.4	18.1	20.5
	Western rye	20.9	19.8	24.0	20.0	19.4	20.8
III	Alfalfa	25.4	23.1	26.1	21.8	22.5	23.8
	Brome	21.4	22.6	–	20.6	20.5	21.3
	Timothy	23.5	22.0	26.5	20.7	21.1	22.8
	Western rye	23.0	22.8	24.1	21.8	20.6	22.5

[1] *Broken in July.*

TABLE IV

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1928

Sub-block	Crop	May 17	June 15	July 10	Aug. 16	Sept. 19	Seasonal average
I	Alfalfa[1]	47.0	44.4	41.0	82.8	160.4	75.1
	Brome[1]	45.8	32.6	–	35.6	102.2	54.0
	Timothy[1]	56.0	62.2	46.2	59.4	137.2	72.2
	Western rye[1]	135.4	46.4	57.8	84.0	131.2	91.0
II	Alfalfa	133.0	92.2	64.6	52.4	57.4	79.9
	Brome	77.2	58.8	–	40.0	52.4	57.1
	Timothy	105.0	88.4	94.0	52.4	58.2	79.6
	Western rye	112.6	100.4	66.8	67.6	55.2	80.5
III	Alfalfa	131.6	57.8	86.6	45.0	60.6	76.3
	Brome	85.0	41.4	–	68.0	50.4	61.2
	Timothy	166.0	102.8	109.4	81.8	59.6	103.9
	Western rye	101.2	79.0	50.7	86.2	62.6	75.9

[1] *Broken in July.*

TABLE V

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1929

Sub-block	Crop	May 14	June 14	July 16	Aug. 16 and 27	Sept. 11	Seasonal average
I	Wheat[1] after alfalfa	20.5	15.6	19.0	18.0	13.2	17.3
	Wheat[1] after brome	20.4	13.5	18.0	15.5	13.2	16.1
	Wheat[1] after timothy	19.8	14.0	19.8	19.4	11.9	17.0
	Wheat[1] after western rye	20.8	13.9	18.0	18.2	10.1	16.2
II	Alfalfa	18.5	11.9	15.8	15.8	10.8	14.6
	Brome	16.0	11.0	15.1	17.3	10.7	14.0
	Timothy	17.1	11.8	18.5	15.8	11.9	15.0
	Western rye	18.0	11.8	17.2	14.2	12.1	14.7
III	Alfalfa	26.5	16.1	20.6	16.1	10.2	17.9
	Brome	24.0	13.9	18.9	15.7	14.9	17.5
	Timothy	23.6	15.1	18.5	17.7	14.8	17.9
	Western rye	24.0	13.2	19.1	16.1	16.2	17.7
	Summerfallow	21.4	14.6	21.9	18.3	21.2	19.5

[1] *First crop of wheat after sod.*

TABLE VI

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1929

Sub-block	Crop	May 14	June 14	July 16	Aug. 16 and 27	Sept. 11	Seasonal average
I	Wheat[1] after alfalfa	122.8	197.8	82.6	112.2	120.2	127.1
	Wheat[1] after brome	58.6	101.4	65.8	40.8	58.2	65.0
	Wheat[1] after timothy	138.0	133.0	57.4	96.0	127.2	110.3
	Wheat[1] after western rye	131.2	113.8	93.4	106.6	153.6	119.7
II	Alfalfa	41.8	33.0	17.4	17.6	8.0	23.6
	Brome	Trace	Trace	10.2	14.8	Trace	5.0
	Timothy	Trace	Trace	33.2	Trace	Trace	6.6
	Western rye	Trace	Trace	12.2	7.4	Trace	3.9
III	Alfalfa	33.0	Trace	28.0	13.8	7.4	16.4
	Brome	Trace	Trace	Trace	Trace	Trace	Trace
	Timothy	Trace	Trace	30.2	Trace	Trace	6.0
	Western rye	Trace	Trace	8.6	Trace	Trace	1.7
	Summerfallow	62.8	84.8	114.6	151.4	217.6	126.2

[1] *First crop of wheat after sod.*

TABLE VII

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1930

Sub-block	Crop	May 12	June 16	July 14	Aug. 22	Sept. 19	Seasonal average
I	Wheat[1] after alfalfa	18.5	19.5	14.9	14.2	17.1	16.8
	Wheat[1] after brome	18.7	19.5	14.5	14.1	17.3	16.8
	Wheat[1] after timothy	18.7	21.6	14.2	15.1	17.4	17.4
	Wheat[1] after western rye	18.7	19.3	14.2	8.8	16.7	15.5
II	Alfalfa[2]	18.5	17.1	11.7	11.5	16.6	15.1
	Brome[2]	16.3	18.5	11.5	12.3	21.2	16.0
	Timothy[2]	17.6	18.7	12.8	8.5	19.1	15.3
	Western rye[2]	15.9	16.2	11.3	9.3	17.5	14.0
III	Alfalfa	19.1	22.0	14.9	14.5	17.6	17.6
	Brome	18.1	21.7	14.9	11.2	18.0	16.8
	Timothy	19.2	20.7	14.2	11.4	18.1	16.7
	Western rye	18.3	20.8	16.5	11.0	16.2	16.6
	Summerfallow	20.4	26.3	20.8	11.4	22.7	20.3

[1] *Second crop of wheat after sod.*

[2] *Broken in July.*

TABLE VIII

NITRATE NITROGEN—POUNDS PER ACRE IN 40-INCH DEPTH—1930

Sub-block	Crop	May 12	June 16	July 14	Aug. 22	Sept. 19	Seasonal average
I	Wheat[1] after alfalfa	112.8	96.0	71.8	147.6	55.0	96.6
	Wheat[1] after brome	78.4	128.4	36.4	95.6	23.2	72.4
	Wheat[1] after timothy	108.4	94.6	33.2	130.4	36.4	80.6
	Wheat[1] after western rye	84.6	71.0	36.4	136.4	39.0	73.5
II	Alfalfa[2]	15.2	34.4	Trace	83.8	45.6	35.8
	Brome[2]	Trace	Trace	None	33.6	5.4	7.8
	Timothy[2]	Trace	Trace	None	81.6	14.2	19.2
	Western rye[2]	Trace	Trace	Trace	53.4	22.2	15.1
III	Alfalfa	11.0	13.2	Trace	23.6	Trace	9.6
	Brome	Trace	Trace	None	Trace	Trace	Trace
	Timothy	Trace	41.4	Trace	55.4	Trace	19.4
	Western rye	Trace	Trace	Trace	35.0	Trace	7.0
	Summerfallow	71.0	42.8	110.0	192.4	60.4	95.3

[1] *Second crop of wheat after sod.*

[2] *Broken in July.*

TABLE IX

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1931

Sub-block	Crop	May 13	June 25	July 24	Aug. 18	Sept. 15 and 25	Seasonal average
I	Wheat[1] after alfalfa	14.7	18.4	19.0	19.4	19.6	18.2
	Wheat[1] after brome	15.6	17.9	18.8	20.3	19.5	18.4
	Wheat[1] after timothy	12.9	19.0	18.1	21.1	17.9	17.8
	Wheat[1] after western rye	14.3	19.5	15.9	20.0	18.6	17.7
II	Wheat[2] after alfalfa	13.5	18.5	18.0	18.7	17.0	17.1
	Wheat[2] after brome	13.0	18.5	19.8	20.5	20.3	18.4
	Wheat[2] after timothy	12.5	20.4	18.3	20.1	18.6	18.0
	Wheat[2] after western rye	11.7	17.8	18.0	20.9	19.0	17.5
III	Alfalfa	12.9	18.6	19.9	23.6	16.4	18.3
	Brome	10.6	17.6	20.2	23.7	20.7	18.6
	Timothy	13.2	21.4	23.7	24.9	21.2	20.9
	Western rye	13.4	20.0	22.5	26.1	21.9	20.8
	Summerfallow	15.0	17.1	23.8	24.6	24.4	21.0

[1] *Third crop of wheat after sod.*

[2] *First crop of wheat after sod.*

TABLE X

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1931

Sub-block	Crop	May 13	June 25	July 24	Aug. 18	Sept. 15 and 25	Seasonal average
I	Wheat[1] after alfalfa	182.8	116.4	73.4	7.6	64.0	88.8
	Wheat[1] after brome	94.2	91.8	38.4	7.2	17.2	49.8
	Wheat[1] after timothy	139.6	108.0	61.4	8.6	45.8	72.7
	Wheat[1] after western rye	151.2	109.0	55.4	8.4	63.2	77.4
II	Wheat[2] after alfalfa	138.2	99.6	45.6	31.2	43.0	71.5
	Wheat[2] after brome	66.4	26.2	23.0	9.4	15.4	28.1
	Wheat[2] after timothy	122.8	94.2	50.4	9.8	40.4	63.5
	Wheat[2] after western rye	139.4	62.8	33.8	9.8	58.4	60.8
III	Alfalfa	51.8	14.6	34.2	21.2	17.2	27.8
	Brome	14.8	17.8	28.2	14.6	17.2	18.5
	Timothy	116.6	34.4	46.4	15.8	13.8	45.4
	Western rye	57.6	97.6	67.8	40.2	61.6	65.0
	Summerfallow	242.2	282.6	214.6	276.8	273.2	257.9

[1] *Third crop of wheat after sod.*

[2] *First crop of wheat after sod.*

TABLE XI

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1932

Sub-block	Crop	May 17	June 17	July 17	Aug. 17	Sept. 17	Seasonal average
I	Wheat[1] after alfalfa	22.7	22.8	19.2	18.7	17.6	20.5
	Wheat[1] after brome	23.3	23.4	17.4	15.5	14.0	18.7
	Wheat[1] after timothy	19.1	20.7	16.3	13.2	13.9	16.6
	Wheat[1] after western rye	21.0	20.3	19.0	13.5	15.3	17.8
II	Wheat[2] after alfalfa	22.2	19.8	18.7	13.4	15.2	17.9
	Wheat[2] after brome	24.1	21.3	16.9	14.3	15.0	18.3
	Wheat[2] after timothy	21.2	22.7	15.8	14.2	13.0	17.4
	Wheat[2] after western rye	21.1	21.2	13.3	11.9	11.4	15.8
III	Alfalfa[3]	20.4	17.1	14.0	12.0	14.1	15.5
	Brome[3]	21.0	20.3	20.5	15.3	18.4	19.1
	Timothy[3]	21.3	20.0	19.1	15.7	18.1	18.8
	Western rye[3 4]	23.2	20.2	22.0	16.7	19.1	20.2
	Summerfallow	21.7	23.5	26.5	20.1	23.7	23.1

[1] *Fourth crop of wheat after sod.*

[2] *Second crop of wheat after sod.*

[3] *Broken in July.*

[4] *Killed by "take-all".*

TABLE XII

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1932

Sub-block	Crop	May 17	June 17	July 17	Aug. 17	Sept. 17	Seasonal average
I	Wheat[1] after alfalfa	57.3	55.6	44.8	5.2	49.6	42.5
	Wheat[1] after brome	72.0	37.7	9.0	7.2	47.8	34.7
	Wheat[1] after timothy	70.2	64.1	6.6	5.2	45.2	38.4
	Wheat[1] after western rye	52.9	57.9	7.2	5.4	42.1	33.1
II	Wheat[2] after alfalfa	65.2	53.6	89.0	50.8	57.2	63.1
	Wheat[2] after brome	34.3	61.2	42.1	7.8	25.8	34.2
	Wheat[2] after timothy	41.5	63.5	42.6	20.0	22.0	37.9
	Wheat[2] after western rye	39.5	79.2	44.8	7.2	33.4	40.8
III	Alfalfa[3]	20.4	17.6	11.4	17.4	56.4	24.6
	Brome[3]	9.9	9.0	11.6	15.0	33.6	15.8
	Timothy[3]	8.3	27.4	44.2	3.3	40.6	24.7
	Western rye[3 4]	38.5	68.7	42.2	35.4	61.2	49.2
	Summerfallow	47.1	92.1	115.6	125.8	136.2	103.3

[1] *Fourth crop of wheat after sod.*
[2] *Second crop of wheat after sod.*
[3] *Broken in July.*
[4] *Killed by "take-all".*

TABLE XIII

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1933

Sub-block	Crop	May 11	June 12	Aug. 16	Seasonal average
III	Wheat[1] after alfalfa	20.9	18.0	11.7	16.9
	Wheat[1] after brome	21.6	18.7	13.2	17.8
	Wheat[1] after timothy	23.7	22.7	14.1	20.2
	Summerfallow	18.2	23.8	20.1	20.7

[1] *First crop of wheat after sod.*

TABLE XIV

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1933

Sub-block	Crop	May 11	June 12	Aug. 16	Seasonal average
III	Wheat[1] after alfalfa	74.6	111.8	39.4	75.3
	Wheat[1] after brome	75.2	96.0	Trace	57.1
	Wheat[1] after timothy	60.0	46.8	Trace	35.6
	Summerfallow	130.0	102.8	194.0	142.3

[1] *First crop of wheat after sod.*

TABLE XV

SOIL MOISTURE—PERCENTAGE (WEIGHTED AVERAGE) IN 40-IN. DEPTH—1934

Sub-block	Crop	May 7	June 8	July 18	Aug. 10	Sept. 7	Seasonal average
I	Wheat[1] after alfalfa	17.7	19.5	16.3	15.5	15.8	17.0
	Wheat[1] after brome	17.3	18.6	15.4	14.8	15.0	16.2
	Wheat[1] after timothy	16.6	16.7	15.5	14.0	14.0	15.4
	Wheat[1] after western rye	16.8	23.1	15.3	14.6	14.5	16.9
II	Wheat[2] after alfalfa	17.1	17.3	16.7	14.4	14.2	15.9
	Wheat[2] after brome	17.6	18.1	15.6	15.1	13.9	16.1
	Wheat[2] after timothy	17.3	18.5	16.6	15.5	13.9	16.4
	Wheat[2] after western rye	17.3	18.1	16.3	14.7	13.9	16.1
III	Wheat[3] after alfalfa	18.1	18.3	17.0	16.1	15.1	16.9
	Wheat[3] after brome	18.1	20.3	18.8	16.1	15.4	17.7
	Wheat[3] after timothy	19.6	20.3	18.1	16.6	15.7	18.1

[1] *Sixth crop of wheat after sod.*
[2] *Fourth crop of wheat after sod.*
[3] *Second crop of wheat after sod.*

TABLE XVI

NITRATE NITROGEN—POUNDS PER ACRE IN 40-IN. DEPTH—1934

Sub-block	Crop	May 7	June 8	July 18	Aug. 10	Sept. 7	Seasonal average
I	Wheat[1] after alfalfa	45.0	44.6	7.6	68.2	41.0	41.3
	Wheat[1] after brome	59.4	40.2	7.4	54.0	39.4	40.1
	Wheat[1] after timothy	41.6	51.8	Trace	62.8	36.4	38.5
	Wheat[1] after western rye	42.6	56.2	Trace	60.4	40.0	39.8
II	Wheat[2] after alfalfa	89.8	81.0	13.6	87.2	83.2	71.0
	Wheat[2] after brome	54.2	62.4	Trace	68.2	28.4	42.6
	Wheat[2] after timothy	46.2	53.4	Trace	66.0	46.2	42.4
	Wheat[2] after western rye	48.4	51.4	Trace	59.0	43.0	40.4
III	Wheat[3] after alfalfa	91.0	77.8	22.4	81.8	72.8	69.2
	Wheat[3] after brome	47.8	40.0	Trace	64.4	12.0	32.8
	Wheat[3] after timothy	59.6	36.2	24.4	61.2	11.8	38.6

[1] *Sixth crop of wheat after sod.*
[2] *Fourth crop of wheat after sod.*
[3] *Second crop of wheat after sod.*

The nitrate nitrogen is expressed as pounds per acre for convenient comparison of the amount present with the amount required per acre by various crops.

It would require too much space to tabulate the corresponding data for each crop and soil depth separately, but examples illustrating the depth distribution under alfalfa and timothy sods and wheat following these sods, and in fallow soil, are shown in Table XVII. The moisture content is expressed as percentage of water-free soil, and the nitrate content as parts of nitrate nitrogen per million parts of water-free soil.

TABLE XVII

MOISTURE AND NITRATE NITROGEN IN SURFACE, SUBSURFACE, AND SUBSOIL. SEASONAL AVERAGES AND AVERAGE OF SEASONS

Sub-block	Depth, in.	1927		1928		1929		1930		1931		1932		Average	
		Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.	Moist. %	Nitrate N, p.p.m.
Alfalfa 1927–28, and Wheat 1929–32															
I	0–6⅔	37.1	21.0	32.2	15.5	23.0	32.1	19.0	23.6	26.6	14.0	24.5	6.6	27.1	18.8
	6⅔–20	26.8	15.2	21.4	4.1	15.6	12.9	14.9	12.4	15.7	9.7	18.2	3.2	18.8	9.6
	20–40	25.7	12.0	20.3	4.6	16.4	1.9	17.4	0.0	17.0	3.7	20.1	2.7	19.5	4.1
Timothy 1927–28, and Wheat 1929–32															
I	0–6⅔	36.8	17.2	30.6	9.4	23.8	22.0	19.9	15.2	24.8	11.7	19.3	6.0	25.9	13.6
	6⅔–20	26.1	17.0	21.3	4.5	15.0	10.5	14.5	7.4	16.6	6.2	16.6	3.1	18.3	8.1
	20–40	25.7	12.4	20.5	5.9	16.0	4.0	18.5	3.4	16.3	4.0	15.7	2.6	18.8	5.4
Alfalfa 1927–32															
III	0–6⅔	38.8	25.4	31.5	6.1	21.6	5.0	21.7	4.8	25.2	10.5	18.8	5.8	26.3	9.6
	6⅔–20	29.3	22.4	23.1	6.5	20.2	1.6	17.3	0.0	18.9	1.7	16.2	2.1	20.8	5.7
	20–40	26.6	16.1	21.6	6.4	15.1	0.0	16.6	0.0	15.6	0.0	13.7	0.7	18.2	3.9
Timothy 1927–32															
III	0–6⅔	35.7	20.2	26.6	5.1	20.2	1.3	17.5	3.2	27.3	7.7	21.3	5.8	24.8	7.2
	6⅔–20	28.5	22.4	21.6	9.4	17.3	0.9	14.8	1.0	21.1	1.4	19.1	1.7	20.4	6.1
	20–40	26.4	14.6	22.3	9.3	17.6	0.0	17.7	1.5	18.6	4.0	17.8	1.6	20.1	5.2
Fallow 1929–32															
—	0–6⅔					30.7	23.6	26.5	28.2	28.8	33.4	31.5	23.7	29.4	27.2
	6⅔–20					19.0	9.5	18.7	6.7	21.1	27.9	25.0	6.8	20.9	12.7
	20–40					16.0	6.8	19.3	2.0	18.3	13.3	19.1	4.1	18.2	6.5

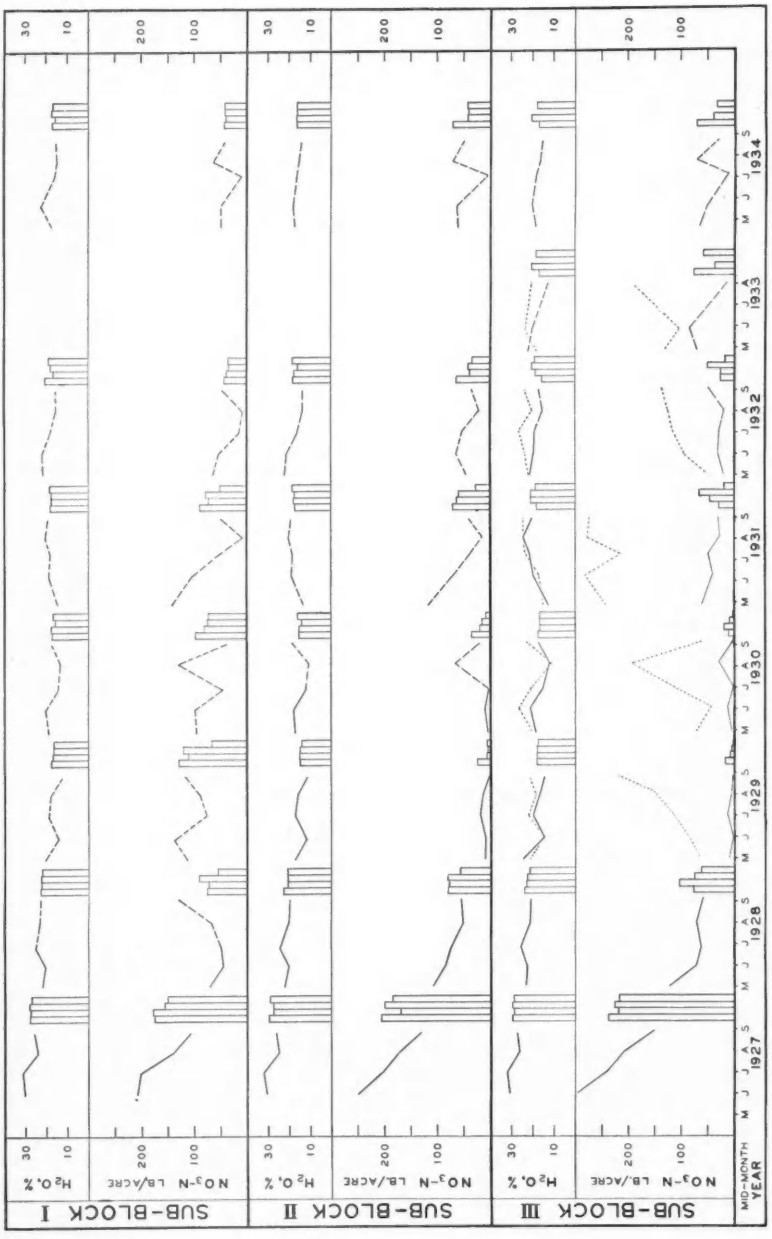


FIG. 1. *Seasonal fluctuations in moisture and nitrate nitrogen to a depth of 40 in. under hay crops (solid lines), under succeeding wheat crops (broken lines), and under fallow (dotted lines). Mean seasonal values under alfalfa, timothy, western rye and brome (in that order from left to right) and under wheat following these sods, shown by histograms.*

The graphs (Fig. 1) show that the moisture and nitrate content of the soil in all three sub-blocks were relatively high in the fallow soil at the beginning of the experiment. After the plots were seeded to alfalfa and grasses in 1927, the moisture and nitrate content of the soil decreased.

Wheat was first grown on Sub-block I in 1929, and the nitrate content of the soil under wheat was for several years generally relatively high following alfalfa, and relatively low following brome grass. Even under the third crop of wheat (1931) following alfalfa, brome, timothy, and western rye grass, the nitrates were generally highest following alfalfa and lowest following brome grass. Afterwards, in 1932 and 1934, the differences were apparently insignificant. The nitrate level of the soil under wheat has a tendency to drop in mid-summer, often reaching its lowest point in July, as shown in the tables and Fig. 1. There was no corresponding decrease in soil moisture, the drop corresponding rather to the period of maximum absorption of nitrates by the growing crop.

After the Sub-block II plots were seeded to alfalfa and grasses in 1927, the nitrate level dropped from year to year, reaching quite a low level in 1929 and 1930, much lower than that of the wheat plots of Sub-block I. After the plots were plowed in 1930 the nitrates increased.

A comparison of the nitrate content of the soil under alfalfa and the different grass sods, from 1927 to 1930, shows that nitrates were generally highest under alfalfa. The tables show that nitrates were highest in the alfalfa plots in three out of four monthly determinations in 1927; in four out of five determinations in 1929; and in the early part of the 1930 season, before the plots were plowed. However, in 1928 the differences between the alfalfa, timothy, and western rye grass plots were apparently insignificant. In three out of the five monthly determinations of the 1929 season there were measurable quantities of nitrate under alfalfa and none or only traces under the grasses. Similarly in May and June, 1930, before the sods were plowed, there were measurable quantities of nitrate in the alfalfa plot soils, but none or only traces in the grass plot soils.

After the sods were broken in 1930 the nitrate content of all plots increased, as shown in Fig. 1. The tables and Fig. 1 histograms show that the nitrate level was generally highest under wheat following alfalfa. Even in 1934 under the fourth crop of wheat, the nitrate level was highest following alfalfa. In the latter part of the 1930 season after the sods were plowed, and throughout the 1931 season when the plots were under wheat, the nitrate level was lower following brome than following timothy or western rye grass. These results confirm the results obtained in Sub-block I.

In Sub-block III, after the plots were seeded to alfalfa and grasses in 1927, the nitrate level dropped from year to year, reaching, as in Sub-block II, quite a low level in 1929 and 1930, much lower than the wheat plot soils of Sub-block I. Although the Sub-block III plots were still under alfalfa and grasses in 1931 and 1932, the nitrate level was generally higher than in 1929 and 1930, and this higher level is attributed to moister seasons. The nitrate content of the soil under wheat in 1933 and 1934 was quite variable.

A comparison of the nitrate content of the soil under alfalfa and grass, from 1927 to 1932, shows that the alfalfa plots were not highest in nitrates as commonly as they were in Sub-block II. However, in 1927 and 1929 the alfalfa plots were generally highest in this respect, and throughout the period from 1927 to 1932 the brome grass plots were generally lower in nitrates than the alfalfa, timothy, and western rye grass plots, as shown in the tables and Fig. 1 histograms. In 1929 and again in 1930 there was never more than a trace of nitrate present in the brome grass plots at the time of the monthly determinations.

After the sods were broken in 1932, the nitrate content of all plots increased to some extent. Under the first and second crops of wheat, in 1933 and 1934, the nitrates were highest following alfalfa, but not consistently lowest following brome grass. No results are given for the wheat plots following western rye grass because these plots were badly infected by root rot before they were plowed in 1932.

As previously noted, the main experiment was supplemented by determinations of moisture and nitrates in nearby fallow plots for the five seasons, 1929 to 1933, to compare these specially favourable conditions with those of the cropped soils. A different fallow plot within the same field was sampled each year. The results are shown in Tables V to XIV and Fig. 1. These data show that the moisture content of the soil was higher in the fallow plots at the end of each of the five seasons than in any of the cropped plots. The nitrates, also, were nearly always higher in the fallow plots than in the cropped plots, and always higher in the latter half of each season.

Water-soluble Phosphorus

There has been much controversy on the subject of determination of available phosphorus, that is, of phosphorus present in the soil that could be readily utilized by plants. Methods of determining available phosphorus have been evolved by Dyer (7), Neubauer and Schneider (16), Winogradsky (31), Truog (26) and others. Neubauer makes use of living plants for the determination. Winogradsky evolved the Azotobacter method. Other investigators employ the method of extraction of soil with different types of solvents which are supposed to simulate the action of roots. Much can be said for these methods, but one must always bear in mind that little as yet is known about the actual form of phosphorus absorbed, and as to how plants extract it from the soil. Any method employed is therefore merely empirical.

In this experiment it was decided to use distilled water for making soil extracts, because it was thought that, whatever the effect of the plants on the soil or its solution, the phosphorus that was present in a water extract would be the most readily available to the plants.

Ignition of the substances obtained by extraction of the soil was carried out to remove the organic matter which would otherwise interfere with the blue colour developed in the determination (20), giving it a yellow tinge. On ignition, however, the organic phosphorus present was changed to the

TABLE XVIII

WATER-SOLUBLE PHOSPHORUS AS PO_4 (P.P.M.) IN SURFACE, SUBSURFACE, AND SUBSOIL—1931

Sub-block	Crop	Depth, in.	May 13	June 25	July 24	Aug. 18	Sept. 15, 25	Horizontal averages	Vertical averages
I	Wheat[1] after timothy	0 – 6⅔	8.1	8.3	9.6	8.9	5.4	8.1	
	Wheat[1] after timothy	6⅔ – 20	3.5	3.7	3.2	4.1	2.8	3.5	
	Wheat[1] after timothy	20 – 40	0.6	0.3	0.2	0.3	0.5	0.4	4.0
I	Wheat[1] after alfalfa	0 – 6⅔	9.3	10.2	10.5	9.9	6.7	9.3	
	Wheat[1] after alfalfa	6⅔ – 20	4.3	3.7	3.1	3.5	2.5	5.4	
	Wheat[1] after alfalfa	20 – 40	0.8	0.6	—	0.2	0.6	0.5	4.4
I	Wheat[1] after brome	0 – 6⅔	9.6	8.1	8.7	10.3	5.3	8.4	
	Wheat[1] after brome	6⅔ – 20	3.6	4.3	3.8	4.1	2.6	3.7	
	Wheat[1] after brome	20 – 40	1.8	0.7	0.2	0.2	0.8	0.7	4.3
I	Wheat[1] after western rye	0 – 6⅔	9.3	9.9	8.7	10.6	7.8	9.2	
	Wheat[1] after western rye	6⅔ – 20	4.1	3.7	3.6	4.8	3.0	3.8	
	Wheat[1] after western rye	20 – 40	0.7	0.4	0.3	0.1	0.5	0.4	4.5
II	Wheat[2] after alfalfa	0 – 6⅔	6.6	9.7	10.6	12.1	5.7	8.9	
	Wheat[2] after alfalfa	6⅔ – 20	3.9	4.5	2.4	—	3.5	3.6	
	Wheat[2] after alfalfa	20 – 40	0.6	0.4	0.3	0.2	0.9	0.5	4.3
II	Wheat[2] after brome	0 – 6⅔	10.2	9.2	10.9	11.9	7.7	10.0	
	Wheat[2] after brome	6⅔ – 20	3.7	4.6	4.5	4.6	3.3	4.1	
	Wheat[2] after brome	20 – 40	1.2	0.5	0.4	0.2	1.0	0.7	4.9
II	Wheat[2] after timothy	0 – 6⅔	8.3	10.1	10.6	9.1	5.8	8.8	
	Wheat[2] after timothy	6⅔ – 20	4.5	5.2	4.0	6.2	4.1	4.8	
	Wheat[2] after timothy	20 – 40	0.5	0.2	0.5	0.7	0.0	0.4	4.7
II	Wheat[2] after western rye	0 – 6⅔	7.6	9.7	9.5	9.5	5.3	8.3	
	Wheat[2] after western rye	6⅔ – 20	3.7	3.2	3.7	4.3	2.6	3.5	
	Wheat[2] after western rye	20 – 40	0.6	0.4	0.6	0.2	1.1	0.6	4.1

[1] *Third crop of wheat after breaking.*
[2] *First crop of wheat after breaking.*

TABLE XVIII—*Concluded*

WATER-SOLUBLE PHOSPHORUS AS PO_4 (P.P.M.) IN SURFACE, SUBSURFACE, AND SUBSOIL—1931—*Concluded*

Sub-block	Crop	Depth, in.	May 13	June 25	July 24	Aug. 18	Sept. 15, 25	Horizontal averages	Vertical averages
III	Timothy	0 – 6⅔	9.0	11.8	11.9	12.2	8.1	10.6	
	Timothy	6⅔ – 20	3.9	4.4	4.7	3.9	2.8	3.9	
	Timothy	20 – 40	0.8	0.5	0.9	0.1	0.5	0.6	5.0
III	Alfalfa	0 – 6⅔	10.0	14.3	12.4	13.5	9.5	11.9	
	Alfalfa	6⅔ – 20	4.1	3.9	4.4	6.0	3.5	4.4	
	Alfalfa	20 – 40	0.9	0.4	0.8	0.7	0.6	0.7	5.7
III	Brome	0 – 6⅔	10.9	11.9	12.2	13.5	8.2	11.3	
	Brome	6⅔ – 20	4.9	4.0	3.7	3.9	3.5	4.0	
	Brome	20 – 40	1.0	0.7	0.8	0.6	1.3	0.9	5.4
III	Western rye	0 – 6⅔	7.9	12.0	11.2	12.8	8.9	10.6	
	Western rye	6⅔ – 20	5.9	4.1	3.4	3.1	3.5	4.0	
	Western rye	20 – 40	1.7	0.5	0.6	0.6	0.5	0.8	5.1
	Fallow	0 – 6⅔	10.4	11.7	11.2	12.6	10.6	11.3	
	Fallow	6⅔ – 20	3.5	2.5	3.2	3.0	1.9	2.8	
	Fallow	20 – 40	0.6	0.3	0.0	0.0	1.0	0.4	4.8

phosphate form and determined as such. A criticism of this procedure is that the plants may not be able to utilize organic phosphorus, and thus the figures may be of little value. However, Whiting and Heck with their work on phytin (28), and others, have shown that plants can make very good use of certain forms of organic phosphorus, directly or indirectly.

The data obtained during the season of 1931 are given in Table XVIII. In considering the results of this season's work one notices that the greatest amount of water-soluble phosphorus is present in the upper six inches of soil, with a gradual decrease downwards. In fact, in the subsoil there is very little water-soluble phosphorus present. It was possible to test the water extracts of a few subsoils directly without first evaporating them and igniting the organic matter. No phosphate was detected in such solutions before ignition, showing that all the water-soluble phosphorus in the subsoil was present in the organic form.

There is no great difference between the figures of the individual plots. However, the concentration of the phosphate in the top layer of the soil seems to be slightly higher under grasses, and in particular under alfalfa, than under wheat, as shown in the vertical average column of Table XVIII. Furthermore, during the actual determination, differences in the intensity of the blue colour developed were clearly seen. Since, however, the differences are slight and are almost within the range of experimental error, one must avoid any very definite conclusion on this point.

There does not seem to be any definite fluctuation in water-soluble phosphorus during the growing season, but the figures show a decrease in the month of September. Only further work can show whether this is a regular phenomenon, or merely peculiar to this season.

Microbial Numbers

The seasonal curves for moisture and numbers of micro-organisms in the surface soil are shown in Fig. 2, and the detailed results in Tables XIX to XXIII.

The numbers of micro-organisms in the Sub-block I plots were determined in 1929 and 1930, under the first and second crops of wheat following alfalfa, timothy, and western rye grass.

The curves show that the moisture content of the surface soil fluctuated greatly in both 1929 and 1930. The numbers of fungi fluctuated but little, and the differences between the different plots are apparently insignificant. The numbers varied from about 10 to 30 thousands per gram. It was observed that large Mucor colonies predominated on the plates inoculated with soil from the alfalfa plots, but not on the other plates (Fig. 3). The numbers of bacteria fluctuated more than the numbers of fungi, and there is some evidence of a correlation between fluctuations in bacterial numbers and moisture, but it is doubtful if the differences between the bacterial numbers in the different plots are significant. The numbers varied from about 4 to 12 millions per gram. The numbers of actinomycetes did not fluctuate very

much until the later part of the season. Through most of the season they varied between one and two millions per gram.

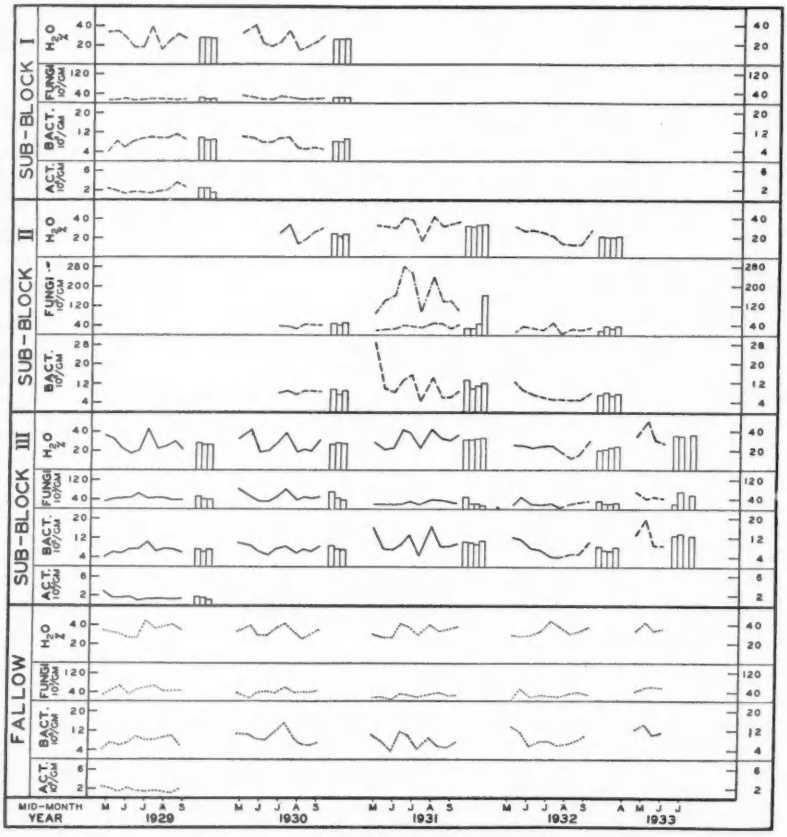


FIG. 2. *Seasonal fluctuations in moisture content and number of fungi, bacteria and actinomycetes in upper 6⅔ in. of soil under hay crops (solid lines), under succeeding wheat crops (broken lines), and under fallow (dotted lines). Fungi in brome plots in Sub-block II, 1931, graphed separately (upper line). Mean seasonal values under alfalfa, timothy, western rye and brome (in that order from left to right) and under wheat following these sods, shown by histograms.*

The numbers of micro-organisms in the alfalfa, timothy, and western rye grass plots of Sub-block II were determined in early July, 1930, just before the sods were plowed up, and throughout the remainder of that season. The numbers under the first and second crops of wheat following alfalfa, brome, timothy, and western rye were determined in 1931 and 1932.

TABLE XIX

SEASONAL FLUCTUATIONS IN MOISTURE AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1929

Sub-block	Crop	May 6	May 20	June 3	June 17	July 1	July 15	July 29	Aug. 12	Aug. 26	Sept. 9	Average
						Moisture, %						
I	Wheat[1] after alfalfa	34.2	34.7	28.7	17.6	20.2	40.4	16.6	26.6	32.0	27.4	27.8
	Wheat[1] after timothy	34.2	36.0	29.5	19.0	18.7	39.4	16.6	24.1	31.6	27.9	27.7
	Wheat[1] after western rye					18.7	39.7	16.1	24.5	33.3	28.1	26.7
III	Alfalfa	36.1	34.6	25.5	19.2	22.5	42.7	20.3	25.5	29.4	21.6	27.7
	Timothy	35.6	30.9	20.8	15.3	20.8	43.3	23.4	24.3	30.0	20.8	26.5
	Western rye					17.9	41.5	23.7	24.9	29.1	21.4	26.4
A-1	Fallow	33.9	31.8	29.9	26.4	25.9	44.0	36.2	38.2	40.4	34.0	34.1
						Bacteria, millions per gm.						
I	Wheat[1] after alfalfa	4.24	8.08	9.41	9.91	9.81	10.67	11.22	10.45	11.22	12.10	9.71
	Wheat[1] after timothy	4.24	9.65	3.07	7.17	10.99	11.02	10.19	9.42	10.95	8.38	8.51
	Wheat[1] after western rye					7.92	8.08	8.18	8.56	12.80	7.52	8.84
III	Alfalfa	5.00	5.65	5.64	8.86	8.11	14.34	5.85	6.81	6.65	5.46	7.24
	Timothy	2.88	6.23	5.64	5.87	7.75	10.40	6.60	7.84	8.01	6.28	6.75
	Western rye					6.86	6.71	7.31	8.11	7.66	6.97	7.27
A-1	Fallow	4.13	7.06	6.01	7.06	9.62	8.27	8.36	9.39	9.87	5.62	7.55
						Fungi, thousands per gm.						
I	Wheat[1] after alfalfa	15	15	21	21	28	24	23	21	21	22	21
	Wheat[1] after timothy	15	16	24	9	15	19	14	18	10	16	16
	Wheat[1] after western rye					12	16	23	15	16	17	16
III	Alfalfa	33	43	54	41	89	52	70	54	53	62	55
	Timothy	27	43	35	53	69	33	31	44	36	25	40
	Western rye					40	47	44	41	22	28	37
A-1	Fallow	27	48	68	33	49	58	66	45	46	46	49
						Actinomycetes, millions per gm.						
I	Wheat[1] after alfalfa	2.66	1.24	1.24	1.52		1.48	1.74	2.25	5.70	2.21	2.23
	Wheat[1] after timothy	2.18	2.56	1.36	1.62		1.24	1.84	1.94	4.60	3.77	2.34
	Wheat[1] after western rye					1.57	1.67	1.68		0.50	2.14	1.51
III	Alfalfa	2.47	1.84	1.89	1.96	1.07	2.09	1.89	1.43	1.48	1.15	1.73
	Timothy	3.25	1.55	1.31	1.50		1.22	1.46	1.33	1.87	1.39	1.65
	Western rye					0.96	0.62	1.30	1.34	0.48	1.78	1.08
A-1	Fallow	2.36	2.08	1.44	2.13	1.60	1.44	1.58	1.38	1.11	1.95	1.71

[1] *First crop of wheat after sod.*

TABLE XX

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1930

Sub-block	Crop	May 7	May 27	June 10	June 24	July 8	July 22	Aug. 6	Aug. 19	Sept. 2	Sept. 15	Average
						Moisture, %						
I	Wheat[1] following alfalfa	33.0	40.5	20.8	19.1	22.7	35.7	14.8	18.4	22.8	30.3	25.8
	Wheat[1] following timothy	32.9	41.8	20.7	18.6	24.6	35.9	15.1	18.5	24.2	31.9	25.8
	Wheat[1] following western rye	32.7	41.3	23.2	20.0	25.9	34.4	14.3	18.9	22.0	29.9	26.3
II	Alfalfa[2]					23.6	36.7	17.2	18.3	26.5	29.6	25.3
	Timothy[2]					26.7	26.4	9.9	18.6	24.3	29.5	22.6
	Western rye[2]					26.0	30.9	16.1	17.6	26.6	30.3	24.6
III	Alfalfa	30.5	39.4	18.9	20.2	25.5	38.5	17.2	20.9	19.9	29.2	26.0
	Timothy	33.0	45.4	18.3	20.0	30.2	39.8	17.7	22.5	19.7	30.9	27.7
	Western rye	33.2	40.7	17.4	18.5	28.1	37.8	21.4	21.2	21.2	31.4	27.1
B–5	Fallow	33.7	41.9	29.5	28.8	35.8	40.9	31.2	24.8	29.9	34.7	33.1
B–5	Fallow (variability)	32.7	37.2	28.6	28.3			30.7			34.3	32.0
						Bacteria, millions per gm.						
I	Wheat[1] following alfalfa	10.16	9.62	10.20	8.68	9.26	11.48	5.33	6.07	6.07	5.62	8.25
	Wheat[1] following timothy	11.00	10.56	7.10	6.76	12.12	10.61	7.05	3.74	5.33	5.50	7.98
	Wheat[1] following western rye	10.80	7.42	6.56	8.27	7.83	10.23	5.21	5.87	6.65	5.20	7.40
II	Alfalfa[2]					7.84	10.12	9.62	9.43	10.00	8.86	9.31
	Timothy[2]					8.32	6.33	6.07	8.01	7.09	7.76	7.26
	Western rye[2]					8.84	10.61	6.61	8.40	9.53	9.37	8.89
III	Alfalfa	12.21	12.07	8.28	4.71	8.08	8.28	6.27	7.69	7.52	9.27	8.44
	Timothy	9.10	6.90	4.92	4.77	6.40	8.26	6.04	7.82	7.38	9.31	7.09
	Western rye	8.60	8.43	6.28	5.52	8.41	8.44	5.31	6.78	4.36	6.86	6.90
B–5	Fallow	11.23	10.71	8.66	7.72	11.48	15.22	8.53	6.54	6.07	6.19	9.23
B–5	Fallow (variability)	10.33	10.43	8.44	8.83			7.84			7.62	8.91
						Fungi, thousands per gm.						
I	Wheat[1] following alfalfa	24	31	16	17	32	29	17	20	19	25	23
	Wheat[1] following timothy	36	24	18	17	30	27	17	20	18	24	23
	Wheat[1] following western rye	44	17	19	17	27	26	21	16	24	17	23
II	Alfalfa[2]					53	58	33	53			46
	Timothy[2]					28	22	31	65	64	39	41
	Western rye[2]					34	13	46	62	62	82	50
III	Alfalfa	114	44	39	52	69	95	54	79	67	85	70
	Timothy	52	68	35	22	47	70	32	45	38	44	45
	Western rye	55	47	25	23	33	79	24	30	30	25	37
B–5	Fallow	35	13	33	43	37	58	39	39	39	43	38
B–5	Fallow (variability)	37	15	39	39			35			44	35

[1] *Second crop of wheat after sod.* [2] *Broken in July.*

TABLE XXI

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1931

Sub-block	Crop	May 11	May 25	June 11	June 24	July 7	July 21	Aug. 11	Aug. 26	Sept. 10	Sept. 22	Average
						Moisture, %						
II	Wheat[1] after alfalfa	32.1	31.2	28.1	42.5	38.8	17.7	41.1	31.9	31.3	34.0	32.9
	Wheat[1] after brome	34.0	31.3	30.3	40.4	37.8	20.2	41.6	32.9	36.2	38.0	34.3
	Wheat[1] after timothy	31.9	31.3	30.0	39.3	34.6	19.6	39.2	29.5	32.0	34.5	32.3
	Wheat[1] after western rye	31.8	30.8	29.4	40.5	39.4	20.5	42.7	31.6	35.6	38.0	34.0
III	Alfalfa	29.3	20.9	23.9	40.6	38.6	18.6	42.7	32.5	27.5	30.7	30.5
	Brome	29.2	21.5	22.9	43.4	36.7	22.8	43.7	32.6	33.1	39.8	32.6
	Timothy	26.5	19.0	21.7	39.7	39.5	21.7	43.3	30.0	32.7	37.0	31.1
	Western rye	29.1	24.3	24.4	42.6	36.8	24.5	41.0	32.7	28.8	36.1	32.0
A–1	Fallow	30.2	26.6	26.6	40.9	37.1	29.5	39.8	34.3	35.0	38.4	33.8
A–1	Fallow (variability)		28.6		40.4	39.4	29.2	40.0	31.4	34.3	37.6	35.1
						Bacteria, millions per gm.						
II	Wheat[1] after alfalfa	36.14	11.60	9.49	14.41	15.87	4.35	19.21	8.85	6.42	5.54	13.19
	Wheat[1] after brome	28.44	10.57	7.43	14.30	17.20	4.75	16.10	4.61	5.67	10.28	11.93
	Wheat[1] after timothy	26.53	7.81	8.17	11.21	12.50	3.45	10.01	2.28	5.51	7.98	9.54
	Wheat[1] after western rye	24.55	8.08	8.16	12.20	13.46	4.96	12.45	7.68	6.38	10.83	10.87
III	Alfalfa	18.39	6.26	6.89	10.31	15.75	4.68	17.43	8.45	8.60	6.35	10.31
	Brome	18.68	8.95	7.54	8.46	13.60	4.89	15.10	8.55	8.64	12.66	10.71
	Timothy	12.76	6.98	7.54	11.24	12.84	4.42	17.90	7.95	10.53	9.25	10.14
	Western rye	15.58	7.70	6.39	7.49	10.26	4.08	16.96	8.14	6.25	9.05	9.19
A–1	Fallow	10.24	6.58	3.38	10.90	10.16	4.80	8.86	6.17	4.48	7.57	7.23
A–1	Fallow (variability)		8.55		11.83	10.43	4.52	8.80	5.04	5.80	6.85	7.73
						Fungi, thousands per gm.						
II	Wheat[1] after alfalfa	10	23	21	21	25	31	53	44	20	18	26
	Wheat[1] after brome	87	132	165	281	257	91	237	139	139	96	162
	Wheat[1] after timothy	19	21	18	36	32	21	37	32	21	22	26
	Wheat[1] after western rye	24	27	38	64	60	34	55	70	43	96	46
III	Alfalfa	47	27	33	49	74	34	65	64	52	36	48
	Brome	14	12	7	9	18	11	29	16	14	16	14
	Timothy	13	17	15	14	23	13	33	29	21	21	20
	Western rye	15	12	10	19	19	16	25	39	21	24	20
A–1	Fallow	16	15	9	30	27	19	32	38	23	25	23
A–1	Fallow (variability)		22		36	31	16	31	35	25	27	28

[1] *First crop of wheat after sod.*

TABLE XXII

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1932

Sub-block	Crop	May 19	June 2	June 19	July 2	July 19	Aug. 2	Aug. 19	Sept. 2	Sept. 19	Av.
					Moisture, %						
II	Wheat[1] after alfalfa	31.8	28.4	26.4	27.0	20.6	16.5	13.4	13.3	26.2	22.6
	Wheat[1] after brome	31.0	27.8	26.6	26.4	21.5	16.6	12.2	12.7	27.6	22.5
	Wheat[1] after timothy	29.5	27.5	26.3	25.2	19.1	16.4	13.2	13.7	25.9	21.9
	Wheat[1] after western rye	29.0	22.9	27.5	23.9	22.2	13.5	13.6	12.3	28.6	21.5
III	Alfalfa[2]	24.6	22.9	22.3	19.0	20.2	16.2	12.8	14.7	27.5	20.0
	Brome[2]	29.0	27.0	25.9	26.2	25.1	21.0	14.4	16.5	30.2	23.9
	Timothy[2]	24.0	25.0	20.4	25.2	21.4	17.6	13.9	14.8	30.3	21.4
	Western rye[2 3]	25.5	23.8	21.7	27.7	24.2	20.0	15.7	16.8	27.5	22.5
	Fallow	29.0	28.0	28.9	33.1	43.1	37.3	29.4	32.5	36.7	33.1
	Fallow	28.9	28.0	29.0	33.3	42.3	39.9	31.6	32.5	37.6	33.7
					Bacteria, millions per gm.						
II	Wheat[1] after alfalfa	7.6	9.3	6.7	7.0	6.1	5.5	5.8	6.3	8.0	6.9
	Wheat[1] after brome	11.8	8.7	7.1	3.7	5.7	5.3	5.7	3.1	7.0	7.4
	Wheat[1] after timothy	20.4	9.9	7.5	6.1	3.9	5.0	4.8	6.0	7.3	7.9
	Wheat[1] after western rye	10.0	8.0	7.0	6.1	5.7	4.6	4.5	4.8	8.7	6.6
III	Alfalfa[2]	14.8	10.9	13.0	6.1	5.0	3.7	5.5	4.5	9.7	8.1
	Brome[2]	14.1	12.4	7.8	8.3	4.3	3.8	5.1	5.1	9.7	7.8
	Timothy[2]	8.8	12.7	4.3	6.0	2.6	3.2	5.0	5.9	9.8	6.5
	Western rye[2 3]	10.3	9.2	5.3	6.0	3.9	4.2	4.3	4.4	10.0	6.4
	Fallow	13.5	12.3	3.2	7.7	7.0	5.1	6.5	7.9	9.7	8.1
	Fallow (variability)	13.8	11.8	8.1	7.6	7.3	6.9	6.8	7.9	10.0	8.9
					Fungi, thousands per gm.						
II	Wheat[1] after alfalfa	9	20	18	17	15	8	14	10	15	14
	Wheat[1] after brome	19	62	52	24	55	5	22	23	40	33
	Wheat[1] after timothy	10	32	21	17	120	6	23	37	26	32
	Wheat[1] after western rye	10	32	24	31	20	3	40	9	35	23
III	Alfalfa[2]	21	68	35	24	43	1	32	26	15	29
	Brome[2]	14	38	11	12	12	4	58	29	30	23
	Timothy[2]	13	49	15	11	10	6	16	23	42	20
	Western rye[2 3]	11	38	8	16	18	13	12		47	20
	Fallow	7	54	20	26	17	17	29	38	22	25
	Fallow (variability)	6	56	19	30	25	24	37	41	34	30

[1] *Second crop of wheat after sod.* [2] *Broken in July.* [3] *Killed by "take-all".*

TABLE XXIII

SEASONAL FLUCTUATIONS IN MOISTURE, AND NUMBERS OF MICRO-ORGANISMS IN UPPER 6⅔ IN. OF SOIL, UNDER ALFALFA, GRASSES, AND FALLOW, IN 1933

Sub-block	Crop	May 3	May 19	June 2	June 17	Average
			Moisture, %			
III	Wheat[1] after alfalfa	33.7	51.0[2]	29.6	26.9	35.3
	Wheat[1] after brome	34.6	48.9[2]	32.2	27.9	35.9
	Wheat[1] after timothy	33.7	47.5[2]	32.3	25.1	34.6
	Fallow	35.3	42.3[2]	31.7	36.1	36.3
	Fallow (Variability)	30.9	42.3[2]	34.5	33.5	35.3
			Bacteria, millions per gm.			
III	Wheat[1] after alfalfa	16.2	17.5	10.6	7.2	12.9
	Wheat[1] after brome	15.0	18.3	7.5	9.8	12.6
	Wheat[1] after timothy	12.9	23.5	8.8	9.1	13.6
	Fallow	12.5	14.3	10.8	10.7	12.1
	Fallow (Variability)	12.4	14.4	9.1	11.0	11.7
			Fungi, thousands per gm.			
III	Wheat[1] after alfalfa	16	19	30	20	21
	Wheat[1] after brome	64	42	61	60	57
	Wheat[1] after timothy	122	59	53	51	71
	Fallow	49	46	58	56	52
	Fallow (Variability)	38	69	64	56	57

[1] *First crop of wheat after sod.*
[2] *Shortly after a shower of rain.*

The moisture content of the surface soil in the different plots fluctuated greatly during the seasons, especially in 1930 and 1931. However, the differences between the different plots or crops in this respect were comparatively small. The moisture varied from about 10 to 37% in 1930, from 17 to 43% in 1931, and from 12 to 32% in 1932.

The numbers of fungi did not fluctuate very greatly except under the first crop of wheat following brome grass in 1931, when the numbers were very much greater than those under the first crop of wheat following timothy, western rye grass, and alfalfa. The numbers varied from about 80 to 280 thousands per gram following brome grass, but only from about 10 to 100 thousands per gram following timothy, western rye grass, and alfalfa. The high counts following brome grass were accounted for mainly by the development of relatively large numbers of small Penicillium colonies on the acid agar plates inoculated with this soil (Fig. 5). This was the first season in which fungi counts were made following the plowing down of brome grass.

There is definite evidence of correlation between soil moisture and numbers of fungi under the first crop of wheat following brome grass in 1931.

Following the plowing down of the Sub-block II sod plots in 1930, it was observed that large Mucor colonies developed rapidly on the plates inoculated with soil from the alfalfa plots, and possibly suppressed the growth of other, slower-growing fungi (Figs. 3 and 4). A similar effect was observed the following year under the first crop of wheat following alfalfa, and in 1932 under the second crop of wheat. The fungi counts (mainly Mucor) were rather small under the first and second crops of wheat following alfalfa, the seasonal average being only 14 thousand per gram of soil under the second.

The numbers of bacteria (including actinomycetes) did not fluctuate very greatly except under the first crop of wheat in 1931, as in the case of the fungi. The numbers varied as a rule from about 6 to 10 millions per gram in 1930, and from about 4 to 10 millions per gram in 1932. However, in the moister season of 1931 the numbers fluctuated a good deal more in all plots. They were extremely high in the spring and afterwards fluctuated between about 4 and 18 millions per gram. The differences between the effects of the different preceding crops were not very significant, but in 1931 especially, the fluctuations in bacterial numbers were obviously correlated with fluctuations in the moisture content of the soil.

The numbers of micro-organisms in the alfalfa, timothy, and western rye grass plots of Sub-block III were determined in 1929, 1930, 1931 and 1932, and in the brome grass plots in 1931 and 1932. In the early part of the 1933 season, when the plots were producing the first crop of wheat, counts were made in the alfalfa, brome, and timothy plots, but not in the western rye grass plots, because these had previously become badly infested with root rot.

Counts were also made of the micro-organisms in the supplementary fallow plots used for the nitrification measurements from 1929 to 1933, in order to compare the numbers present in the cropped plots of the main experiment with the numbers present under conditions known to favour increase in moisture and accumulation of nitrates. During the four seasons, 1930 to 1933, two sets of composite samples were taken from the fallow plots as a rule, in order to study the variability of the composite samples.

The moisture content of the surface soil in the different plots fluctuated considerably, but there was less fluctuation in 1932 than in the other four years. The differences between the cropped plots were small as a rule, but, in every case except the relatively wet season of 1931, the moisture content of the fallow plot soil was higher in the latter part of each season than that of the cropped plots.

The numbers of fungi fluctuated considerably during the five seasons, 1929 to 1933, varying commonly from about 10 to 80 thousands per gram of soil. During three out of four of the hay crop years, the fungi counts were generally higher in the alfalfa plots than in any of the grass plots; this applies to 1929, 1930, and especially to 1931, but not to 1932. Under the first crop

of wheat, in 1933, it was observed that, as in the cases of Sub-blocks I and II, Mucor colonies predominated on the plates inoculated with soil from plots in which alfalfa had been turned under (Fig. 3). The counts were relatively low in these plots, thus agreeing with the results obtained in Sub-block II.

The number of bacteria (including actinomycetes) fluctuated considerably during the five seasons, 1929 to 1933, in which counts were made. During the first four of these, the Sub-block III plots were under alfalfa and grass. The numbers varied from about 4 to 12 millions per gram of soil in 1929 and 1930. In the moister season of 1931 the numbers varied from about 4 to 18 millions per gram, and from about 2 to 14 millions in 1932. Under the first crop of wheat, in 1933, counts were limited to May and June and varied from about 6 to 18 millions per gram of soil. The differences between the effects of the different crops (alfalfa, brome, timothy, and western rye grass) were not very significant. Neither was there a significant difference between the effects of the different crops and fallow. However, the seasonal fluctuations in bacterial numbers were obviously correlated with fluctuations in the moisture content of the soil in all five seasons.

The numbers of actinomycetes did not fluctuate very much during the one season, 1929, when counts were made in the alfalfa and grass plots of Sub-block III. They varied usually between one-half million and two millions per gram of soil. The counts obtained in a nearby fallow plot in 1929 did not fluctuate very much either.

During the season of 1930 the numbers of amoebae were determined by the dilution plate count method, in the alfalfa and timothy plots of Sub-block III, and in the fallow plots. Throughout this season amoebae were nearly always found in the $\frac{1}{1000}$ dilution plates but not in the $\frac{1}{10.000}$ dilution plates.

Distribution of Bacteria and Fungi in the Soil Profile Layers

In the main experiment, soil samples for microbial counts were taken to a depth of $6\frac{2}{3}$ in., or to the plow depth approximately, as it is generally believed that most of the microbial activity takes place in this upper layer of soil. However, a supplementary experiment was carried out during the season of 1932 in order to obtain a better idea of the distribution of bacteria and fungi in the Edmonton soil profile layers. The samples were taken from nearby cultivated and old sod plots. Large holes were dug in these plots to a depth of 36 in., and successive samples were obtained at later dates by digging back into the wall of soil, and thus taking samples well back in the unexposed soil each time. The plots were sampled five times during the season, and the counts obtained are given in Table XXIV.

Although in the cultivated plot the surface layers generally contained most bacteria, the deeper samples gave fairly high counts in both the cultivated and sod plots. The fungi counts in the cultivated plot were generally highest in the surface layer, but in the sod plot the deeper samples gave fairly high counts.

TABLE XXIV

SEASONAL FLUCTUATIONS IN NUMBERS OF MICRO-ORGANISMS AT VARIOUS DEPTHS IN CULTIVATED AND GRASS SOD EDMONTON SOIL, IN 1932

Depth, in.	Horizon	May 30	June 30	July 30	Aug. 30	Sept. 30	Average
		Fungi in cultivated soil, thousands per gm.					
0 – 3	A_1	35	8	10	15	22	18
3 – 6	A_1	30	6	6	4	5	10
6 – 12	A_1	3	2	3	3	6	3
14 – 22	A_2	2	2	1	5	5	3
22 – 29	B_1	1	6	0	3	5	3
29 – 36	B_2	0	0	1	2	5	2
		Fungi in grass sod soil, thousands per gm.					
0 – 3	A_1	19	15	38	44	7	25
3 – 6	A_1	12	7	13	10	4	9
6 – 12	A_1	13	4	5	5	4	6
14 – 22	A_2	6	19	7	19	21	14
22 – 29	B_1	4	18	17	12	25	15
29 – 36	B_2	9	18	37	21	14	20
		Bacteria in cultivated soil, millions per gm.					
0 – 3	A_1	13.0	6.5	4.2	6.7	11.3	8.3
3 – 6	A_1	10.4	4.8	4.1	5.8	2.8	5.6
6 – 12	A_1	3.2	1.1	1.5	1.9	2.5	2.0
14 – 22	A_2	5.2	0.7	2.2	2.9	3.7	2.9
22 – 29	B_1	3.9	0.9	2.3	2.6	4.1	2.8
29 – 36	B_2	2.6	0.5	4.6	2.0	4.4	2.8
		Bacteria in grass sod soil, millions per gm.					
0 – 3	A_1	9.0	0.9	2.9	6.3	3.5	4.5
3 – 6	A_1	5.2	1.7	2.5	2.4	2.0	2.8
6 – 12	A_1	4.8	0.8	2.1	2.8	2.2	2.5
14 – 22	A_2	8.6	4.1	2.9	6.7	3.3	5.1
22 – 29	B_1	5.4	1.3	1.7	2.6	3.8	3.0
29 – 36	B_2	5.8	1.5	0.5	5.7	4.4	3.6

TOTAL NITROGEN

Table XXV shows the total nitrogen content of the alfalfa, brome, timothy, and western rye grass plot soils and of the wheat plots in the three sub-blocks. The nitrogen determinations were made once a year, on samples of surface, subsurface, and subsoil taken for the nitrate determinations. The surface soil in every case contained most nitrogen and the subsoil least. Although the samples analyzed were composites, there was considerable variation from year to year which cannot reasonably be attributed to actual changes in soil composition, but must arise from random sampling variations. The maximum variation in the nitrogen content of the composite samples of surface soil

TABLE XXV

TOTAL NITROGEN CONTENT OF EDMONTON PLOT SOILS, DETERMINED YEARLY

Sub-block	Replicates	Crop	Depth, in.	June 15, 1927	May 17, 1928	May 14, 1929	May 12, 1930	May 13, 1931	May 17, 1932	May 7, 1934
I	1–7–12–14	Timothy	0–6⅔	0.63	0.58	0.54	0.58	0.52	0.52	0.58
			6⅔–20	0.26	0.22	0.33	0.29	0.20	0.20	0.28
			20–40	0.10	0.09	0.08	0.10	0.09	0.08	0.09
I	2–8–11–13	Alfalfa	0–6⅔	0.54	0.53	0.47	0.56	0.54	0.53	0.52
			6⅔–20	0.25	0.21	0.26	0.31	0.24	0.24	0.31
			20–40	0.10	0.10	0.09	0.10	0.08	0.08	0.07
I	3–6– 9–16	Brome	0–6⅔	0.57	0.58	0.55	0.58	0.52	0.51	0.54
			6⅔–20	0.23	0.22	0.22	0.31	0.24	0.24	0.32
			20–40	0.10	0.12	0.08	0.12	0.08	0.08	0.06
I	4–5–10–15	W. rye	0–6⅔	0.58	0.56	0.53	0.59	0.46	0.44	0.58
			6⅔–20	0.23	0.30	0.33	0.31	0.32	0.26	0.30
			20–40	0.10	0.10	0.10	0.10	0.11	0.11	0.08
II	1–6–12–15	Alfalfa	0–6⅔	0.57	0.59	0.64	0.62	0.60	0.59	0.64
			6⅔–20	0.23	0.24	0.31	0.22	0.26	0.25	0.31
			20–40	0.10	0.10	0.13	0.11	0.09	0.08	0.08
II	2–7–9–16	Brome	0–6⅔	0.65	0.53	0.61	0.61	0.61	0.59	0.62
			6⅔–20	0.23	0.23	0.27	0.26	0.22	0.23	0.37
			20–40	0.10	0.10	0.16	0.10	0.13	0.10	0.08
II	3–8–10–13	Timothy	0–6⅔	0.62	0.56	0.51	0.60	0.58	0.56	0.59
			6⅔–20	0.22	0.24	0.22	0.29	0.27	0.26	0.28
			20–40	0.10	0.09	0.20	0.10	0.08	0.07	0.08
II	4–5–11–14	W. rye	0–6⅔	0.62	0.56	0.58	0.59	0.60	0.59	0.63
			6⅔–20	0.29	0.23	0.32	0.28	0.30	0.26	0.31
			20–40	0.11	0.09	0.22	0.11	0.09	0.08	0.08
III	1–8–10–15	Timothy	0–6⅔	0.70	0.65	0.65	0.66	0.66	0.65	0.69
			6⅔–20	0.29	0.28	0.41	0.30	0.31	0.26	0.36
			20–40	0.11	0.10	0.29	0.12	—	0.08	0.08
III	2–5–11–16	Alfalfa	0–6⅔	0.70	0.66	0.72	0.66	0.70	0.68	0.72
			6⅔–20	0.32	0.22	0.32	0.28	0.27	0.28	0.37
			20–40	0.12	0.09	0.18	0.10	0.08	0.08	0.08
III	3–6–12–13	Brome	0–6⅔	0.70	0.74	0.69	0.70	0.68	0.67	0.73
			6⅔–20	0.25	0.30	0.25	0.26	0.28	0.27	0.28
			20–40	0.11	0.10	0.20	0.10	0.08	0.08	0.09
III	4–7–9–14	W. rye	0–6⅔	0.69	0.69	0.69	0.66	0.64	0.63	
			6⅔–20	0.32	0.30	0.42	0.27	0.38	0.27	
			20–40	0.11	0.10	0.20	0.10	0.12	0.09	

throughout the seven yearly determinations was 0.12%. This happened in the samples from the brome grass plots of Sub-block II. The variations showed no definite trend downwards or upwards throughout this period of years.

Discussion

Soil microbiological activity was measured in this experiment in order to study some underlying causes of the comparative effects of alfalfa, brome,

timothy, and western rye grass on the yield and nitrogen content of succeeding wheat crops.

It was previously shown at Edmonton that relatively large amounts of nitrate are produced in Edmonton black soil after clover sod is plowed down (32, 33), and a number of investigators, including Lyon, Bizzell, and Wilson at Cornell (14), had shown that greater amounts of nitrate are produced in the soil following crops of clover and alfalfa, or after these crops have been plowed under, than following cereals and grasses. However, the effect of alfalfa on the yield and nitrogen content of succeeding wheat crops, as well as on nitrification had not been investigated previously at Edmonton.

Investigations by Albrecht (1) and others have shown that nitrification is depressed by timothy. It was therefore desirable to compare the effect on nitrification of timothy with that of brome and western rye grass, as well as alfalfa. It was also important to compare nitrification under wheat following these different grasses and alfalfa, in relation to the yield and nitrogen content of the wheat crop.

When the previously fallowed soil was seeded to alfalfa and grasses, the moisture and nitrate content of the soil were reduced, and generally remained at a relatively low level until the sods were plowed up. In previously reported experiments (17, 32, 33) it was found that whereas fallow land at Edmonton usually contained most soluble nitrate nitrogen, and soils supporting the ordinary grain crops contained intermediate amounts, the perennial crop soils, and particularly the grass crop soils, usually contained least. In the experiments reported in this paper the nitrates were reduced to a very low level or disappeared entirely in the grass and alfalfa plots in the drier seasons, but increased considerably in the moister seasons. The nitrate content of the alfalfa plot soils was generally greater than that of the grass plots, and the brome plots were generally lower in nitrates than the other grass plot soils.

The nitrate content of the grass and alfalfa plot soils was generally lower than that of the wheat plots, especially in the drier seasons. The high nitrate content of the fallow plots may be explained in large measure by the higher moisture content of the fallow plots and the lack of absorption by crops, but the explanation for the higher nitrate content of the wheat plot soil as compared to the perennial plot soil is not very clear. The moisture content of the grain plots was not appreciably greater than that of the perennial crop plots. The cultivation before seeding grain may have improved the physical condition of the soil and thus increased nitrification. However, some other explanation based upon crop characteristics may account for the difference.

When alfalfa plots were plowed, nitrification proceeded more rapidly, or nitrates accumulated to a greater extent, than in the plowed grass plots. The greater nitrate content of the soil under wheat following alfalfa was observed for a period of three or four years following the plowing of the sods in separate sub-blocks plowed up two years apart. The greater nitrate production can be explained most satisfactorily, probably, on the basis of the higher nitrogen content of alfalfa residues as compared to grass residues.

The greater nitrification following alfalfa has an important effect on the composition of crops, as shown by the fact that the protein content of wheat in this experiment was considerably higher following alfalfa than following the grasses for the period of three or four years (19). In other experiments on gray, wooded soils of Alberta, it has been shown that the application of ammonium salt fertilizers at seeding time has very little effect on the protein content of wheat, but that the plowing down of clovers produces a marked increase in the protein content of the succeeding wheat crop, probably because the nitrogen of the clover becomes available gradually during the growing season (35). It is interesting to find that this effect of legumes on the protein content of succeeding wheat crops is produced even in the relatively fertile Edmonton black soil.

Not only were the brome grass plots generally lower in nitrates than the other grass plots, but the wheat plots following brome were also generally lower in nitrates than following timothy or western rye grass. This lower nitrification was reflected in a smaller absorption of nitrogen by the wheat crop and a lower protein wheat following brome than following the other two grasses. The reason why the nitrogen of brome grass residues is less available than that of the other grasses is not definitely known. The brome grass residues may be decomposed with greater difficulty by soil micro-organisms, although the average carbon to nitrogen ratio of the residues is no wider than that of timothy, in these experiments, and but slightly wider than that of western rye grass. According to Russell (22, p. 313) and others, the nitrogen of soil organic matter can appear as nitrate only if it exceeds a certain critical amount relative to carbon; and when the proportion of carbon is greater, the nitrogen remains as complex protein. This critical ratio is usually 12, or less, of carbon to one of nitrogen, and until the excess of carbon is lost by decomposition, nitrate will not appear in the soil, or will be absorbed as rapidly as it is produced. Collison and Conn (3), Heukelekian, Waksman and Skinner (10, 27) and others have shown that cellulose is decomposed in the soil to a large extent by fungi, which require a source of available nitrogen.

The absorption of nitrogen by the wheat crop indicates the rate of soil nitrification, as stated in the first paper of this series (19). However, a better indication is given by both absorption of nitrogen and accumulation of nitrate in the soil. In these experiments the greatest absorption occurred under wheat following alfalfa, where nitrate accumulated to the greatest degree, and the least under wheat following brome, where nitrate accumulated to the smallest degree.

The tendency of the nitrate content of the soil under wheat to drop in mid-summer may be explained satisfactorily by the fact that this corresponds with the period of maximum absorption of nitrates by the growing crop. The effect of the growing crop is further shown by a comparison with the fallow plots. The nitrate content of the fallow soil fluctuates, but does not show this tendency to drop in mid-summer, and the fallow plots were relatively high in both nitrate and moisture at the end of each season.

Several investigators, including King and Whitson (12) and Russell (21), showed that the period of loss of nitrate in the soil corresponded with the period of rapid growth of crops. King and Whitson (12), Jensen (11), Russell (21), and Whiting and Schoonover (29) found that the most active period of production and accumulation of nitrates was the late spring and early summer, and that there was an increase in nitrification in the early autumn, following a period of low nitrification in the late summer. However, neither early summer nor fall increases in nitrification were obtained consistently in the fallow plots at Edmonton, and nitrate accumulation was not interfered with by crop growth on these plots. These results tend to support the contention of Lemmermann and Wichers (13), who believe that physical factors affect the nitrification process, and contend that there is not sufficient evidence to show a direct periodic influence of the time of the year on the life activities of the organisms.

The different crops of this experiment apparently utilized about the same amounts of soil moisture, as the differences between the moisture content of the different plots were not great. However, in Sub-block III the oldest stand of alfalfa in the experiment was plowed up in July 1932, and the mositure content of the soil was lower throughout that season in these alfalfa plots than in any of the grass plots. Duly (6) found that old stands of alfalfa reduced to an appreciable extent the moisture at the lower depths.

Broadly speaking, an increase in moisture is usually accompanied by an increase in nitrate accumulation, under a given crop, but there is no very close correlation between fluctuations in moisture and nitrate nitrogen. This lack of correlation may be explained in part by the fact that the moisture and nitrate determinations were made only once a month. Furthermore, it should be kept in mind that in all cases except the summerfallow, crops were growing on the plots and utilizing both nitrates and moisture. However, it will be observed that the general level of nitrates under alfalfa and grasses in Sub-blocks II and III dropped from season to season between 1927 and 1929, inclusive, as the moisture content of the soil decreased. Also, on comparing the dry seasons of 1929 and 1930 with the moister soil conditions of 1931 and the early part of 1932, one notices that the nitrate content of the continuous alfalfa and grass plots of Sub-block III was higher in 1931 and 1932 (Fig. 1), showing that an increase in moisture stimulates nitrification.

The distribution of nitrate nitrogen and moisture in the different depths is shown in Table XVII, in the form of seasonal averages (and averages of several seasons) for enough of the treatments and years to give a good idea of its nature. It will be observed that the average distribution of nitrate is rather similar whether the plots are supporting wheat (Sub-block I), alfalfa (Sub-block III), timothy (Sub-block III), or fallow, in spite of the large differences in the total amount present. The surface often contains about twice as high a concentration as the subsurface, and the subsurface about twice the subsoil. Nitrate is readily soluble and moves downward in the soil to a considerable extent by diffusion and movement of soil moisture. Occasion-

ally greater concentrations were found in the lower depths, and this may be accounted for by leaching or diffusion, and utilization near the surface by plants or micro-organisms. It is reasonable to believe that most of the nitrification occurs in the surface and that the nitrate in the subsoil has been brought down by diffusion or leaching from the upper layers.

The numbers of fungi and bacteria, as determined by the plate count method, did not fluctuate very much in certain plots and seasons, but fluctuated greatly in others. As in previous experiments (17), the fluctuations in nitrate nitrogen did not correspond closely to fluctuations in numbers of fungi, bacteria, or actinomycetes. The fluctuations are undoubtedly affected by food supply and other factors. Moisture is probably one of the most important of these factors, as there is evidence of a correlation between soil moisture and numbers of micro-organisms, especially in the case of bacteria.

The work of Cutler, Crump, and Sandon (5), and of Thornton and Gray (25), showed that the bacterial numbers vary considerably from one day to the next, and even from hour to hour. Superimposed on these fluctuations are the great seasonal changes in numbers observed by Russell and Appleyard at Rothamsted (23), and by other workers elsewhere. One object of the experiments reported in this paper was to measure these larger seasonal changes.

The importance of bacteria in the decomposition of plant residues has been generally recognized for a longer period of time than the importance of other soil micro-organisms. Of late years experimenters such as Waksman, Skinner, and Heukelekian (10, 27) have come to the conclusion that a large proportion of the decomposition and synthesis of organic matter within the soil is brought about by soil fungi. Increasing attention has been given lately to the competition between different groups of soil micro-organisms, and between higher plants and soil micro-organisms, for nutrients present in the soil.

Hiltner, many years ago, and others who have more recently investigated the influence of higher plants on micro-organisms, have shown that micro-organisms tend to accumulate close to the roots of plants. It has been demonstrated by Conn and others that the numbers of bacteria determined by the plate count method represent only a small fraction of the total number present in the soil (2).

Dr. F. J. Greaney, Professor N. James, Dr. J. E. Machacek, and Dr. W. L. Gordon found at Winnipeg, Manitoba, that the total bacterial counts (including actinomycetes) of replicate plates had an abnormal variance, deviating from what was expected according to the laws of probability. They found, however, that the distribution of the total number of fungi in replicate plates agreed well with the theoretical*. We therefore investigated the variability of replicate plate counts from given composite samples of soil**. The differences in counts between parallel plates should arise solely from random sampling variations in the number of micro-organisms withdrawn

* *Private communication.*

** *With the assistance of Dr. J. W. Hopkins of the National Research Council, Ottawa, to whom grateful acknowledgment is made.*

from suspensions in each aliquot, and should therefore occur in accordance with Poisson's law, provided that all the organisms (or a constant proportion of them) deposited on the plates grow or produce colonies, and provided there are no discrepancies arising from deficiencies in manipulation, incubation, etc. In order to determine whether the actual counts conformed to the theoretical requirements, it was necessary to calculate the index of dispersion appropriate to the Poisson distribution. When this was done it was found that in the counts of bacteria (including actinomycetes) the probability of chance deviations of the magnitude obtained was remote. In counts of fungi the agreement was better, but still unsatisfactory on the whole, although the replicate plate counts of some plots throughout certain seasons were within the limits of probable chance deviation. It is quite likely that more of the replicate plate counts of fungi would have fallen within the limits of probable chance deviation if the medium had not contained protein, which tends to encourage the development of spreading proteolytic fungi. Czapek's protein-free medium (8) was used in the experiments previously referred to, at Winnipeg. The closer approximation of the fungal counts to the theoretical requirements, in spite of the known defects of the plate count method for fungi, may help to explain why the fungi counts seem to be of greater significance in these experiments than those for bacteria.

Fortunately, no abnormal variations were encountered in the parallel determinations of moisture, nitrate, and phosphate; for a given sample of soil there was close agreement between duplicates.

The abnormal variance in replicate plate counts from the same sample of soil makes it difficult to establish significant differences in counts between different soil samples. The actual variation between different samples has been calculated from the results of counts of duplicate composite samples from the fallow plots in 1930 to 1933, shown at the bottom of each section in Tables XX to XXIII. For bacteria (including actinomycetes) the standard error within dates varied from zero to a maximum of 0.7 millions per gram of soil (with the exception of one date in 1932) during all four seasons. Thus, to be statistically significant when the difference between duplicate counts was greatest, within or between dates, the plus or minus difference from the mean of the duplicate counts would require to be 1.4 millions per gram.

For the counts of fungi, the standard error within dates varied from 0.3 to 2.1 thousands per gram of soil in 1930, from 0.3 to 2.5 thousands in 1931, from 0.3 to 4.2 thousands in 1932, and (with one exception) from zero to 5.5 thousands in 1933. Here, statistically significant variations from the mean of duplicates, within or between dates, would need to be 4 thousand per gram in 1930, 5 thousand in 1931, 8.5 thousand in 1932, and 11 thousand in 1933. Although the standard error of microbial counts of plots other than fallow cannot be calculated, it may be assumed, not unreasonably, that the sampling errors of the other plots are similar to those of the fallow plots.

It was rather disappointing to find that the differences between numbers of bacteria in the alfalfa, brome, timothy, western rye grass, and fallow plots

were not very significant. In previously reported experiments at Edmonton (17) covering a period of only two seasons, the seasonal average number of bacteria was greatest in fallow land and least in grass land, corresponding to the highest and lowest average moisture content of the soil. However, in the experiments reported in the present paper this correlation between seasonal average number of bacteria and seasonal average moisture content of soil often did not exist, in spite of the correlation between fluctuations of soil moisture and numbers of bacteria.

A spring maximum of bacterial numbers was observed in about half of the seasonal curves. Spring maxima were observed in the earlier experiments at Edmonton (17), and both spring and fall maxima have been observed by other workers in more humid regions.

There is evidence of a correlation between soil moisture fluctuations and fungal count fluctuations under the first crop of wheat following brome grass in 1931, but otherwise there is less evidence of correlation between fungal counts and moisture fluctuations than there is between bacterial counts and moisture. As in earlier experiments at Edmonton (17), some evidence of correlation between fluctuations in numbers of fungi and bacteria was observed, but these correlations were not obtained consistently.

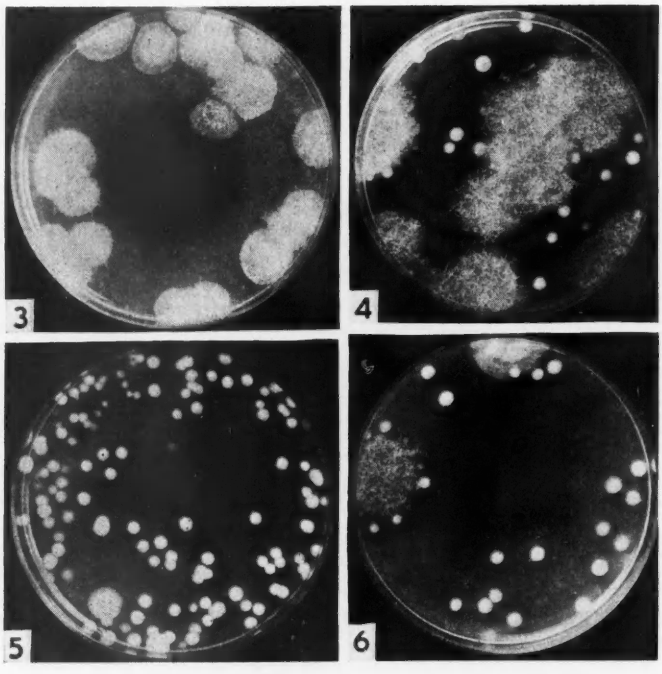
A fungus colony developing on a plate medium may represent a small bit of mycelium or a mass of mycelia, a single dormant spore or a mass of spores, or a mixture of mycelia and spores, and consequently the plate count method has often been considered of doubtful value for determining numbers of fungi present in a soil. But in spite of the known defects of this method the results seem to be of greater significance, in these experiments, than those for bacteria. Some interesting relationships between the different crops and crop residues and the numbers and kinds of fungi developing on the plates were observed.

The numbers of fungi were generally higher in the alfalfa plots than in the brome, timothy, or western rye grass plots, but the differences between these grass plots were apparently insignificant.

Under the first crop of wheat it was repeatedly observed that large Mucor colonies predominated in the alfalfa plot soil plates and the counts were relatively low (Fig. 3). In a soil treated with alfalfa roots, according to Martin (15) the predominant types of mould were Mucor, Rhizopus and Alternaria. The low counts do not necessarily indicate a small number of fungi or absence of other forms, because it was necessary to make the counts after only two days of incubation, on account of the rapid development of the Mucor colonies. If counting was delayed the plates became so overgrown by the fungus that the separate colonies could not be distinguished (Fig. 4). Probably other, slower growing types of fungi were present in the soil, but on account of the rapid growth of Mucor on the plates, no opportunity was given them for development. This opinion is confirmed by the fact that during the later part of the season, when the Mucor colonies did not spread with great rapidity on the plates, Penicillium colonies appeared on the third day of incubation, as illustrated in Fig. 4. The great development of Mucor

may be explained by the fact that Mucor is a fast-growing proteolytic fungus, and alfalfa residues are rich in protein. When incorporated with the soil, alfalfa apparently forms a suitable medium for this fungus.

Under the first crop of wheat in 1931, the brome grass plot soils gave by far the highest counts of fungi, and these appeared on the plates mainly as small Penicillium colonies (Fig. 5). These may have been stimulated in the soil



FIGS. 3 – 6. FIG. 3.—*Plated from soil after plowing down alfalfa. Note rapid development of Mucor after two days incubation. No other fungi are seen.* FIG. 4—*Plated from soil after plowing down alfalfa. Note the growth of Mucor and the development of other colonies (mainly Penicillia) after three days incubation.* FIG. 5—*Plated from soil after plowing down brome. Note great numbers of colonies, mainly Penicillia.* FIG. 6.—*Plated from soil after plowing down timothy. Note small numbers of colonies as compared with Fig. 5.*

by the incorporation of a large quantity of carbonaceous organic material of a character peculiar to brome grass. Apparently it is not a greater utilization or exhaustion of soil moisture by brome that is responsible for the poorer yield or quality of wheat crops following this grass, as the moisture contents of the soil of the different plots growing crops did not show any great differences. This would throw some doubt on the popular idea among farmers at the present time that brome causes poor growth of subsequent crops of wheat by drying out the land.

The numbers of actinomycetes, as determined by the plate count method, did not fluctuate very greatly during the one season in which they were determined, and the differences between the different plots were apparently insignificant. As in previous experiments (17), the actinomycetes were fairly numerous, but considerably less numerous than the bacteria. However, it is doubtful whether plate counting methods for actinomycetes are reliable, as a considerable number of bacterial colonies develop on the medium and often cannot be distinguished readily from actinomycetes colonies.

Apparently the numbers of amoebae present in Edmonton soil are relatively small compared, for example, to the numbers present in Rothamsted soils, where the interrelationship of soil protozoa and bacteria has been extensively investigated. At Rothamsted the number of amoebae present was usually more than 100,000 per gram of soil (24, p. 90) whereas at Edmonton the number was less than 10,000 per gram. The numbers of bacteria in the Edmonton soil, as determined by plate count methods, were usually much smaller than the numbers present in Rothamsted soil, and a few daily counts made in 1929 indicate that daily fluctuations are not nearly as marked in the Edmonton soil.

In the supplementary experiment on the distribution of bacteria and fungi in the Edmonton soil profile layers, it was found that the deeper layers gave surprisingly high counts of bacteria in both cultivated and sod plots, and that the deep samples from the sod plot gave surprisingly high counts of fungi, as shown in Table XXIV. However, the activity of the micro-organisms cannot be proportional to these counts, for there is relatively little activity in the subsoil.

The quantities of water-soluble phosphorus never exceeded about 14 parts per million in any of the plots throughout the one season in which this determination was made, and there were no pronounced fluctuations during the the growing season. The greatest quantities were found in the surface soil and the least in the lowest depth samples. The differences between different plots were not very significant. Most, if not all, of the water-soluble phosphorus is probably in organic combination, and may not be readily available to higher plants, but it has been shown by other investigators that plants can make use of some organic phosphorus compounds (28, 30).

A comparison of the seven successive yearly total nitrogen determination figures for the alfalfa, brome, timothy, and western rye grass plot soils, and wheat plot soils following these sods, shows that there was considerable variation from year to year. In no series of successive nitrogen determinations was the maximum variation less than 0.05% in the surface soil, and it was as great as 0.15% in one series. The variations cannot reasonably be attributed to actual changes in soil composition, and must arise mainly from random sampling variations. Each sample analyzed was a composite prepared from samples of four separate plots, which might account for a greater variation than would be obtained if the composites had been prepared from single plots. Nevertheless, these results emphasize the importance of sampling

error in any study of changes in the total nitrogen content of a soil produced by a given treatment. The changes in a period of seven years as measured in this experiment are obviously insignificant.

Acknowledgment

Acknowledgment is made to Mr. C. Obee and Mr. A. D. Paul, for assistance at various times.

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RESEARCHES ON DROUGHT RESISTANCE IN SPRING WHEAT

I. A MACHINE FOR MEASURING THE RESISTANCE OF PLANTS TO ARTIFICIAL DROUGHT[1]

By Cyril B. Kenway[2] and H. B. Peto[3]

Abstract

The "chinook machine", designed and constructed by Dr. O. S. Aamodt, was reconstructed to provide economy of operation by the installation of a return air conductor, and uniform conditions for all plants exposed to treatment at one time, by means of a travelling belt to which the pots are fastened.

Since the publication of the results of the drought investigations conducted by Aamodt and his associates (2, 3), numerous experimental data on field and laboratory tests have been assembled. This paper is concerned only with a description of alterations and improvements made in the "chinook machine" originally described by Aamodt (1).

The original machine was characterized by two serious defects. In the first place, the warm air escaping from the end of the chamber into the greenhouse resulted in unduly high greenhouse temperature and excessive cost. The other defect was concerned with the impossibility of subjecting all the pots in one batch to similar conditions. Both temperature and air current velocity varied from one part of the chamber to another.

In order to avoid overheating the greenhouse and to reduce the cost of operation, a return air conductor was installed. The conductor was provided with horizontal partitions to reduce eddying, and with small adjustable intake and outlet openings for the purpose of ventilation. As a result of this change, the time required to bring the chamber up to a temperature of 110° F. was reduced from about two hours to 8 or 10 min., the consumption of electricity was greatly reduced, and the greenhouse temperature was only slightly raised during the tests.

Essentially uniform conditions for all pots in one batch were insured by the installation of a travelling belt to which containers for the pots were attached. The original floor was slightly lowered and covered with 24-gauge galvanized iron sheets to make a suitable base for the moving pots. At each end of the chamber an 8-in. sprocket was installed, the one next to the fan being fastened to the floor, while the other was connected directly to a gear reduction assembly. The latter was adapted from an automatic coal stoker, and had a gear reduction of 1800 to 1. The gear assembly was driven by a $\frac{1}{4}$ h.p. electric

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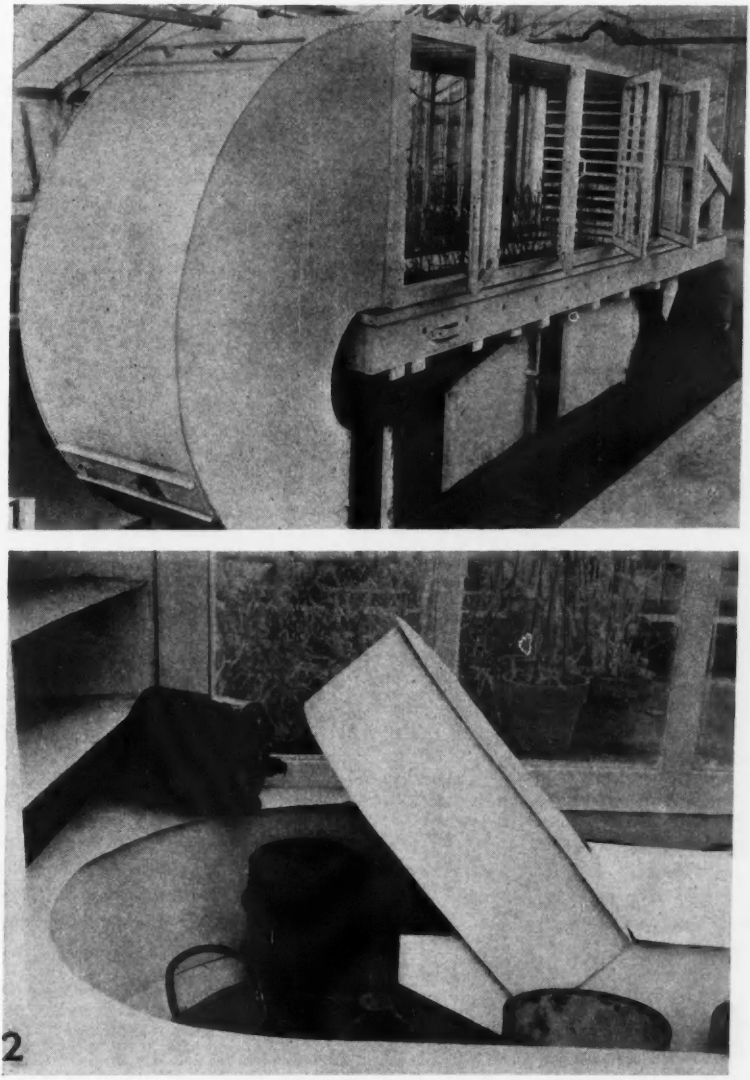
[3] *Graduate Assistant.*

motor. An endless steel chain taken from the conveyor belt of a threshing machine was fitted to these sprockets. Galvanized cylindrical cans, supported by small furniture castors, were fastened to the chain by door springs (Fig. 2).



FIGS. 1 and 2. 1. *General view of chinook machine.* 2. *Interior view of chinook machine, illustrating pot containers and the method of their attachment to the endless chain.*

During treatment, the experimental material is in continuous motion, each pot making one complete revolution in six minutes. By this means, essentially uniform conditions for all material in any one batch are provided. The only drawback to this change is that the capacity of the machine is reduced from about 40 to 25 pots.

The alterations described above are illustrated in Figs. 1 and 2.

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AN APPARATUS FOR MEASURING THE "FLASH" THERMAL DEATH POINT OF MICROSCOPIC ANIMAL ORGANISMS AND ITS USE WITH OVA OF *ASCARIS LUMBRICOIDES*[1]

BY W. E. SWALES[2] AND DAROL K. FROMAN[3]

Abstract

A method of measuring "flash" thermal death points of microscopic animal organisms is described. By means of the devised apparatus the time of exposure can be varied from 0.5 to 0.1 second, and the temperature can be estimated with relative accuracy. In a sample determination, the single-celled ova of *Ascaris lumbricoides* (porcine origin) were all destroyed at a temperature of 68° C. in an exposure of 0.44 second.

The free-living stages of certain animal parasites have been shown by numerous workers to be extremely resistant to the action of chemical substances. It has been shown, however, that the destruction of such organisms can usually be accomplished by thermal means. Hot water is commonly used in applied parasitology to destroy the eggs of nematodes and the cystic stages of certain protozoa parasitic in man and higher animals, particularly when these organisms are present in buildings and enclosures as potential sources of infection. Water and aqueous solutions of chemicals at high temperatures have been proved to be highly efficacious as ovicides. In agricultural parasitology such agents are highly practical in prophylactic measures against nematodes of the families Ascaridae, Heterakidae, Oxyuridae and Trichinellidae, and against the many species of coccidia that infest mammals and birds. The McLean County system of sanitation for the prevention of roundworm and other infections of pigs relies upon the destruction of the ova in breeding pens by the use of hot water.

In devising practical methods of destroying infection in breeding pens for pigs in eastern Canada, it became evident that exact knowledge regarding the efficacy of methods was not readily available. Roberts (5) had tested the resistance of ova of *Ascaris lumbricoides* against hot water at temperatures ranging from 50 to 100° C., and had found that they were destroyed at 70° C. in from one to two seconds, and at 80, 90, and 100° C. in a fraction

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Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que. (with financial assistance from the National Research Council of Canada), and the Department of Physics, Macdonald College, Que.

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of a second. Wharton (6) had shown that all eggs of *A. lumbricoides* were killed at 70° C., and Ogata (3) had shown that they were destroyed in one second at 70° C., in more than 50 sec. at 65 ° C., and in more than 45 min. at 50° C.

It seemed highly desirable, for practical purposes, to set a tentative "flash" thermal death point for the ova of *Ascaris* and for similar organisms, so that standard methods of sanitation would have a definite basis. Consideration of factors that would influence the contact of water with the egg under practical conditions indicated that the time factor would have to be very short, particularly when the work was carried out in cold weather. Thus it was considered that some point below half a second would serve as a practical time standard for the measurements of thermal death points of free-living stages of animal parasites. A description of the technical methods employed by Ogata was not available. Roberts had made his measurements by drying the ova on glass slides and exposing them to hot water by rapid immersion; it was apparent that this method would involve protection of the eggs to some extent through the heat capacity of the glass slide, particularly in very short exposures, and thus was not considered suitable as a standard method.

In our investigation, an apparatus has been devised that appears to eliminate errors to a large extent.

Description of Apparatus and Methods

The problem of maintaining a microscopic egg or oöcyst at any desired temperature for a short, but measurable, time was solved by projecting an excess of the organisms into a vertical stream of heated water; the stream carried the organisms into a large volume of water. It was assumed that the time required by an individual organism to attain the temperature of the stream was equal to the time of cooling in the cold water bath, and that this time factor was negligible; thus the time of exposure was taken to be the time the organism remained in the stream. This assumption is supported by the example that if the surface of an egg of *Ascaris lumbricoides* at room temperature be suddenly raised to 60° C. and maintained at that temperature, an approximate calculation shows that the centre of the egg will be above 59.9° C. within 1/1000 sec.

Let h_1 be the distance the water has fallen under the action of gravity to the point at which the organism is introduced into the stream. Let h_2 be the total height through which the water falls, the distance h_2 being measured from the surface of the water in a releasing cup, shown as *F* in Fig. 1, to the

FIG. 1. *A diagram of the apparatus as arranged in a test.*

A. *Vessel for hot water, equipped with knife heater and thermometer.* B. *Vessel for suspension of organisms.* C_1. *Inner glass tube.* C_2. *Outer glass tube, forming hot water jacket.* D. *Vessel of water for cooling organisms.* E. *The apparatus for selecting central stream* (S) *and discarding splashed or delayed organisms (shown enlarged in* Fig. 1c). F. *Release cup for forming vertical stream* (S). G. *Galvanometer.* H. *Finely drawn capillary tube for injecting suspension of organisms.* J_1 and J_2. *Thermocouple junctions.* K. *Tube conveying hot water.* P_1 and P_2. *Motor-driven stirring paddles.* R. *Variable resistance.* S. *Vertical stream.* T. *Sealed glass tube containing thermocouple wires.* FIG. 1b. *Enlarged diagram of thermocouple junction* (J_1).

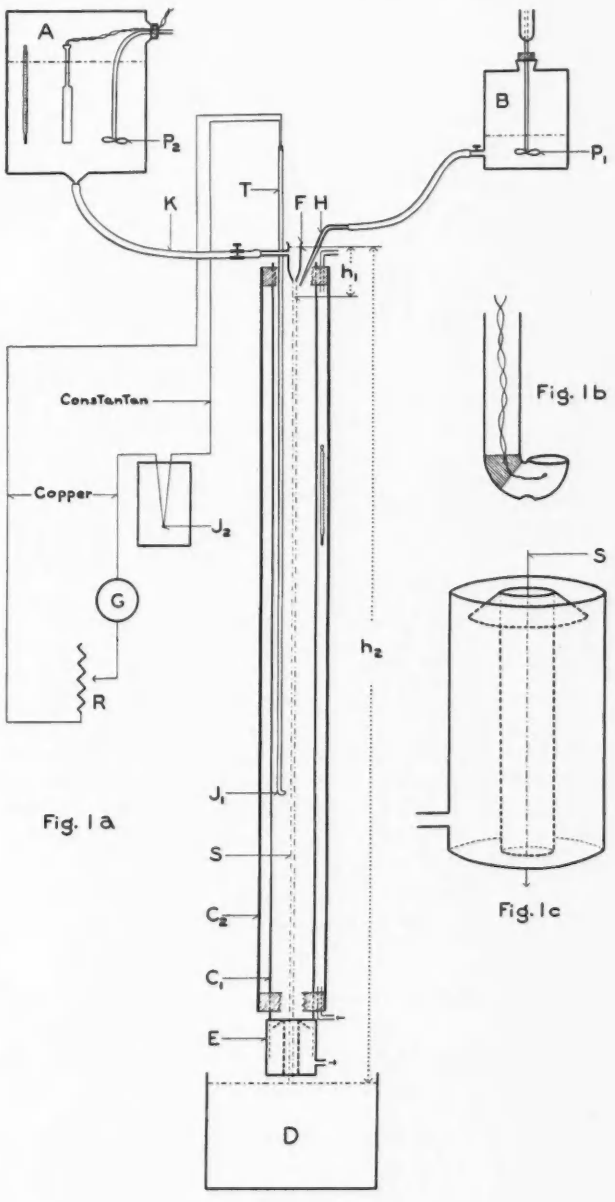
A
B
P2
P1
K
T
F
H
h1
ConsTanTan
Copper
J2
G
R
h2
J1
S
C2
C1
E
D
Fig. 1a
Fig. 1b
S
Fig. 1c

FIG. 1

surface of the cold water. Then the velocity (v_1) of the stream at the point of entrance of the organisms is given by

$$v_1 = \sqrt{2gh_1}$$

where g is the acceleration of gravity. The velocity of the stream at the surface of the cold water is

$$v_2 = \sqrt{2gh_2},$$

hence the time of exposure is

$$t = \frac{2(h_2-h_1)}{v_1+v_2} = \sqrt{\frac{2}{g}}(\sqrt{h_2}-\sqrt{h_1}). \qquad (1)$$

Experience shows that $h_1 = 7$ cm. is a convenient distance. Taking $h_1 = 7$ cm. and $g = 981$ cm./sec.^{2}, Table I has been calculated from Equation (1) for certain values of h_2.

TABLE I

THE TIME (t) REQUIRED FOR AN OBJECT TO FALL UNDER GRAVITY FROM THE POINT OF ENTRANCE OF THE ORGANISMS INTO STREAM (S) TO COLD WATER SURFACE

t, sec.	h_1, cm.	h_2, cm.
0.1	7.0	21.6
0.2	7.0	50.0
0.3	7.0	86.2
0.4	7.0	132.3
0.5	7.0	188.2

The apparatus was arranged as shown in Fig. 1. Water was heated to any desired temperature by means of the immersed "knife-type" heater in a 6-litre vessel, A, and was stirred by a motor-driven paddle (flexible shaft). The water was allowed to flow through a tube, K, at a speed required to maintain a constant level in a cup, F. The water fell under gravity from an aperture in the bottom of the cup into an 8-litre container, D, nearly filled with cold water. The organisms under study were kept at room temperature in a 1-litre bottle, B. A small stream of water carrying these organisms was projected into the main stream, S, from a finely drawn glass tube, H, at a measured depth, h_1, below the water level in the cup. The water at this point was falling with the speed a freely falling body would attain in falling a distance h_1 (Torricelli's theorem). The time of exposure to the high temperature was calculated by means of Equation (1) from the observed values of h_1 and h_2 (Fig. 1).

In order to keep the temperature of the stream constant during the fall, it was surrounded by two glass tubes, C_1 and C_2. Hot water was circulated between these tubes. The inner tube was 4.4 cm. outside diameter and about 140 cm. long, with a 2-mm. wall. The outer tube was 8 cm. outside

diameter and about 125 cm. long, with a 2-mm. wall. It was found that practically no change in temperature occurred along the falling stream if the jacket temperature was kept within 10° C. of the stream temperature.

Owing to a small amount of splashing, some water collected on the inside of the tube C_1 and ran down the walls. This water was discarded by means of the collecting can, *E*. The flange on the top of the inner cylinder of this can prevented any splashed water from falling into the vessel, *D*, and, in fact, only the central stream was admitted into vessel *D*.

The temperature of the water at any desired point in the stream was measured by means of copper-constantan thermocouple junction, J_1, projecting from the end of a glass tube, *T*. This junction is shown enlarged in Fig. 1b. The ends of the wires were painted with "Glyptal" after being soldered, and the end of the glass tube was shaped so that the junction was continuously surrounded by the water in the stream. The small hole in the bottom of the catch-cup allowed free circulation of the water. The temperature of the stream was measured at three definite levels immediately before and after each test. The thermocouple was kept completely out of the stream during a run while the container *D* was in place. Thus no organisms which might have been held momentarily on the end of the tube *T* could get into *D*. The second junction, J_2, was kept at 0° C. in a mixture of ice and water contained in a Dewar (or Thermos) flask. The temperature was read by means of a dead-beat galvanometer, *G*. (Any linear galvanometer with a sensitivity of about 0.02 microamps per division is suitable.) The temperature sensitivity was about 2.5 divisions per degree C. The galvanometer scale was calibrated by immersing junction J_1 in a beaker of water, whose temperature was taken with a standard mercury thermometer. This calibration was checked frequently, and any necessary small corrections to the galvanometer readings were made by adjusting the resistance, *R*. The resistance used was a 0 to 10,000 ohm box, variable in steps of one ohm. (A 1000 ohm rheostat would be suitable.) An alternative method of reading the temperature would be the use of a potentiometer and null-point galvanometer.

Rough estimates of the probable errors in the temperature measurements were made from the variations of temperature along the stream during the time of a run, as well as from the precision of the thermocouple system. The temperature can be measured and maintained constant within a probable error of about 0.1° C.

Use of the Apparatus. Tests on Single-celled Eggs of *Ascaris lumbricoides* (Porcine Origin)

For the purpose of demonstrating the apparatus, eggs of *A. lumbricoides* were used. Mature worms were collected in abattoirs as they were removed from the intestines of newly killed pigs and were promptly immersed in physiological saline at 38 to 40° C. In the laboratory they were cleaned and

placed in fresh saline in an incubator at body temperature. The eggs produced by these worms, during the first 24 hr. only, were used, after being washed and stored in water at 4° C. On each day on which tests were made the eggs were allowed to slowly regain room temperature, and large numbers were then suspended in container *B*. The suspension was kept in continual motion by means of the motor-driven stirring paddle, P_1.

In these tests the original apparatus was used, t having been found to have the value of 0.44 sec. after measurements of h_2 had been made. As the original object was to test the effect of exposures to certain temperatures for a period of less than $\frac{1}{2}$ sec., this point of 44/100 sec. was considered as a satisfactory "flash point" for preliminary tests.

The apparatus was further prepared by almost filling vessel *A* with water and heating it to a temperature slightly above the point it was required to test; the top of the container was sealed for the duration of each test. Trials of the vertical stream and the mixture of stream and suspension of eggs were made in order to ensure that the centre of the stream would fall through the aperture in the cup *E*. The space between tubes C_1 and C_2 was then filled with hot water (at a temperature within 10° C. of that of the proposed test), which continued to flow by tap pressure at a controlled rate throughout each test. The vessel of cold water, *D*, (16 to 18° C.) was placed near the apparatus, and the main vertical stream and the small stream of egg suspension were allowed to fall into a temporary container in place of vessel *D*. Temperatures of the stream were then read in units on the galvanometer scale by one operator, as the other operator placed the thermocouple junction, J_1, in the vertical stream at three points. If the temperature was satisfactory and if the three points did not vary more than 0.1° C., a test was immediately taken by placing vessel *D* at the base of the stream for a period of about 30 sec., during which time one operator stirred the cold water in vessel *D*, and the other ascertained that the apparatus was working correctly. At the end of this period, vessel *D* was quickly replaced by the temporary vessel. The temperatures of the stream at the same three points were again taken, and the operation of the apparatus was stopped. If the subsequent examination of the six records of temperature showed that the stream had remained at a constant temperature or that the highest did not vary from the lowest reading by more than 0.2° C., the test was recorded as satisfactory. By a simple process of sedimentation, the eggs that had fallen into vessel *D* were recovered, and several thousands were placed in "Syracuse" watch glasses, where they were distributed evenly over the bottom and allowed to dry. A similar number was recovered from the suspension in vessel *B*, and these unexposed eggs were dried on another watch glass for control observations. As soon as the eggs had dried, each set was labelled, placed in a moist chamber, and kept at 27° C.

The criterion of survival adopted was the ability of each egg to form an active embryo. After two weeks, 500 eggs were examined microscopically as a preliminary estimation of results. In examinations made two months

after each test, it was found that in some preparations, which showed no survival at the first reading, a few eggs would have recovered enough to form living embryos. Therefore, in the data given in Table II, the results of examining 500 eggs two months after each test are used as the final figures of percentage survival.

Seventeen satisfactory tests have been made to determine the "flash" thermal death point of single-celled ova of *A. lumbricoides*, the results being shown in Table II and Fig. 2.

In Fig. 2 the diameters of the circles around the plotted points show, approximately, the standard errors in the temperature determinations. The lengths of the vertical lines represent the expected standard errors due to sampling (500 eggs) in estimating the percentage survival.

TABLE II

THE EFFECT OF EXPOSURE OF SINGLE-CELLED OVA OF *A. lumbricoides* TO HOT WATER FOR 44/100 SECOND

	Temp., °C.	Exposed eggs, % living embryos	Control, % living embryos	Per cent survival
1	56.0	99.0	99.0	100.0
2	60.5	99.2	99.0	100.0
3	61.5	99.0	99.0	100.0
4	63.0	97.7	98.2	99.5
5	63.75	98.7	98.8	100.0
6	64.0	93.9	98.2	95.6
7	64.5	93.5	98.8	94.6
8	64.5	88.8	98.0	90.6
9	65.4	90.4	98.8	91.5
10	65.5	55.1	98.0	56.2
11	65.6	52.6	98.8	53.2
12	66.2	13.9	98.2	14.2
13	66.2	5.6	99.0	5.7
14	66.4	30.5	98.8	30.9
15	66.8	7.1	98.8	7.2
16	67.4	2.6	98.2	2.6
17	68.0	0.0	99.0	0.0

Discussion

The results of these determinations form a surprisingly steep curve. The critical death point of single-celled eggs of *Ascaris lumbricoides* is, apparently, between 67 and 68° C., although the majority are destroyed at points between 66 and 67° C., when exposed for a period of 0.44 sec. These results are not in complete accord with those of previous workers, in that they show that all the eggs are destroyed in 0.44 sec. at a lower temperature than those reported. We believe that the difference may be due to the fact that our method eliminates such factors as the heat capacity of the object used to hold the eggs during exposure in previous methods, and thus ensures that the egg reaches and is maintained at the temperature for a known "flash" period. As the

results became evident, every effort was made to detect possible mechanical errors, but none was found; and the method has now been described as a practical means of determining "flash" thermal death points for similar animal organisms, and possibly for plant organisms.

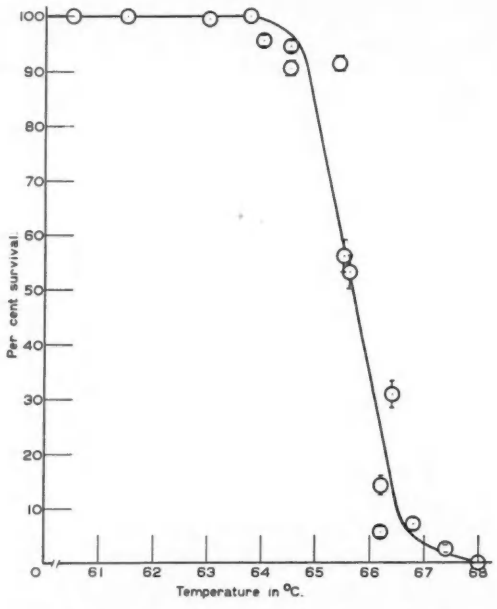

FIG. 2. *The resistance of single-celled ova of Ascaris lumbricoides to exposure in hot water for 0.44 sec. Circles and vertical lines represent standard errors.*

The time factors used in such measurements of "flash" thermal death points cannot replace the standard time of 10 minutes used by biologists in estimating the thermal death point of organisms, but accurate estimations of the effects of exposures of less than half a second would have direct application in designing measures of control against pathogenic organisms. For example, "flash-point" measurements would have value in extending such tables as those published by Reinhardt and Becker (4) on the effect of temperature on infectivity of coccidial oöcysts.

It is felt that these preliminary results justify publication of the general description of the apparatus and methods. In building a similar apparatus, other workers will no doubt make modifications according to their facilities, and for this reason no description has been given of minor details. Some difficulties may be experienced with the vertical stream, but adjustment of the form of the outlet will overcome excess splashing.

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FOOD OF DUCKS AND COOTS AT SWAN LAKE, BRITISH COLUMBIA[1]

By J. A. Munro[2]

Abstract

A study of the autumn food habits of ducks and coots at Swan lake, British Columbia, based on the examination of 136 stomachs and on co-related field work, indicates the following conclusions: pond ducks had eaten 78% plant material, 12% *Chara*, and 10% animal organisms; the food of diving ducks was 65% plant material, 31% *Chara*, and 15% animal matter; while that of coots was 97% *Chara* and 3% plant material. *Chara* is the dominant growth in the lake. It is produced in unlimited quantities so that the food requirements of coots do not seriously compete with those of ducks.

Introduction

In order to obtain precise information on the food relations between coots and ducks as it applied to one particular area, a study of bird populations and their food was undertaken at Swan lake, near Vernon, British Columbia. This body of water is typical of certain marshy lakes which are known to be the common nesting grounds for various species of ducks and for the American Coot, *Fulica americana americana*, as well as being seasonal concentration places for the coot. In the autumn, when the local coot population has been increased many times by an influx of migrants, the total number is impressive. The coots associate in large flocks on open water, where they are more conspicuous than are the scattered diving ducks and Baldpate, *Mareca americana*, which usually accompany them, while the population of pond ducks, which frequent inshore shallows and sheltering marsh growth, may escape observation altogether. Thus the disproportion in numbers of ducks and coots may appear greater than it actually is, although the autumn concentration of coots may greatly outnumber the total of all species of ducks present.

The investigation was carried on at intervals during the years 1932 to 1937 inclusive and comprised population counts of waterfowl at different seasons, the collection and identification of plants and animal food organisms, and the collection for the purpose of stomach analyses of ducks and coots during the autumn months, when greatest concentration occurred. As the study has been a local and seasonal one, the conclusions reached regarding the food relations are not necessarily applicable to spring and summer periods, nor to lakes in which different physical, chemical, and biological conditions exist.

Numbers of Waterfowl

The figures given in enumerations of waterfowl as set forth in Table I represent exact counts in some instances; in others it was necessary to rely upon estimates made as carefully as conditions would permit. It is believed

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TABLE I

ENUMERATIONS OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA

—	Common Mallard	Gadwall	Baldpate	American Pintail	Green-winged Teal	Blue-winged Teal	Cinnamon Teal	Shoveller	Redhead	Ring-necked Duck	Canvas-back	Scaup Ducks†	American Golden-eye	Barrow's Golden-eye	Buffle-head	White-winged Scoter	Ruddy Duck	Total of ducks	American Coot
May 16, 1932	20	–	2	2	–	14	20	4	200	–	2	4	2	3	–	10	40	323	1000
Sept. 15	200	50	20	100	300	200	*	3	400	–	–	–	–	20	30	5	500	1828	800
20	100	6	6	100	250	10	–	1	50	–	–	7	–	6	30	–	200	766	765
Nov. 11	–	–	–	–	–	–	–	–	30	–	–	400	50	–	100	–	–	580	700
May 11, 1933	10	2	–	–	4	13	7	–	118	–	5	8	–	17	57	–	67	295	150
Sept. 30	300	5	50	400	400	50	–	–	10	–	15	20	–	1	10	–	8	–	2500
Nov. 1	100	4	15	200	75	2	–	–	60	12	200	60	10	–	100	1	50	889	6000
4	200	–	150	250	100	–	–	75	50	10	50	300	10	–	200	–	–	1395	6000
17	1000	–	30	40	–	–	–	8	1500	–	100	500	10	–	30	5	–	3223	4000
Sept. 29, 1934	350	50	300	150	250	100	–	75	1	5	–	–	–	–	–	–	30	1311	3000
Oct. 4	100	30	500	300	275	6	–	10	–	–	20	15	–	–	8	–	–	1264	400
18	100	50	50	50	50	20	–	10	–	1	150	–	–	–	6	–	1	488	2000
Sept. 15, 1935	90	–	10	–	20	30	–	10	70	7	–	–	–	12	–	–	150	399	750
23	75	10	30	–	10	30	–	10	25	12	1	–	–	–	–	10	30	243	1500
May 2, 1936	3	3	–	–	–	–	11	5	119	3	–	6	–	3	20	–	104	277	644
20	14	–	1	–	–	6	4	6	222	–	–	20	–	3	14	–	22	312	81
June 4	3	2	–	–	–	2	8	4	211	5	–	6	1	–	–	–	3	245	61
Sept. 12	202	5	35	–	101	133	–	1	299	–	–	–	–	3	1	–	161	941	1500
21	130	35	30	6	80	28	–	–	70	–	–	–	–	–	–	–	60	439	1000
June 12, 1937	32	3	–	–	–	16	14	6	214	–	–	14	–	1	–	–	20	320	108
Oct. 1	250	10	400	140	800	140	–	30	40	5	4	6	–	–	5	–	20	1850	2500
8	38	14	250	10	50	28	–	6	15	–	–	5	–	6	–	–	12	434	2500
June 4, 1938	28	13	13	–	–	8	6	20	143	–	1	–	–	–	–	–	20	252	86

Not recorded above—Old-squaw; Sept. 30, 1933, 1; Nov. 1, 1933, 2.
Hooded Merganser: April 4, 1932, 3; Nov. 1, 1933, 3.
American Merganser: May 26, 1932, 4; May 2, 1936, 11.

**Autumn counts of Blue-winged and Cinnamon teal are combined.*

†Scaup ducks are not recorded specifically.

that a fair degree of accuracy in estimating waterfowl populations can be achieved through constant practice and that the margin of error is constant enough so that the figures have comparative value.

The following species and numbers of birds were used in the stomach examinations:—

Common Mallard— *Anas platyrhynchos platyrhynchos*	12	Redhead— *Nyroca americana*	8
Gadwall— *Chaulelasmus streperus*	5	Ring-necked Duck— *Nyroca collaris*	5
Baldpate— *Mareca americana*	9	Canvas-back— *Nyroca valisineria*	1
American Pintail— *Dafila acuta tzitzihoa*	10	Greater Scaup Duck— *Nyroca marila*	1
Green-winged Teal— *Nettion carolinense*	10	Buffle-head— *Charitonetta albeola*	6
Blue-winged Teal— *Querquedula discors*	2	Old-squaw— *Clangula hyemalis*	2
Cinnamon Teal— *Querquedula cyanoptera*	5	Ruddy Duck— *Erismatura jamaicensis rubida*	9
Shoveller— *Spatula clypeata*	6	American Coot— *Fulica americana americana*	45

These were collected during the months of September, October, and November in the years 1933 to 1937. As no appreciable difference was apparent in the food taken in different years it has not been considered necessary to treat year groups separately.

Study of the stomach material was made at the Pacific Biological Station, Nanaimo, British Columbia, and the identification of aquatic insects was largely the work of the Director, Dr. W. A. Clemens. For this co-operation and for the use of the laboratory facilities of the Biological Station, the author wishes to make grateful acknowledgment.

Description of Lake

Swan lake, slightly over three miles long with a maximum width of about 1,000 yards, is situated in a narrow valley of farm lands which, in orchard and pasture, extend up the open hills to the east and west. The maximum depth is 24 ft. (June 24, 1922); the bottom is partly humus on clay and partly marl, with smaller areas of sand and gravel. B. X. creek, which enters the south end of the lake, is the chief tributary, and the spring flood water from this source causes a rise of water level which reaches the peak in early June. This supply usually fails before midsummer as a result of water diversion for irrigation; subsequently the water level rapidly falls through evaporation, so that portions of the lake bottom in the shallows may be

exposed by September. At this time also, sheltered portions of the lake are usually covered with the "bloom" of blue-green algae, which disappears with the onset of colder weather in the autumn. The shores, except for a few stretches of open, muddy beach, are encircled with a belt of bulrushes and cat-tails which widens into marshes at either end. On the east and west shores, between high water mark and the edge of cultivation, is a discontinuous growth of alder, willow, black haw, mountain birch, and several small stands of trembling aspen.

Analyses of Stomach Contents

The results of the examination of the contents of waterfowl stomachs, shown in Tables II to IV, are here briefly summarized.

The food of 59 pond ducks of eight species collected during the months of September, October, and November comprised 78% vegetable matter, 12% *Chara* branches and oospores and 10% animal organisms, chiefly molluscs and insects. Seeds of aquatic plants, with *Scirpus* first and *Potamogeton* second, were the most important both in respect to time of occurrence and average percentage volume.

The food of 32 diving ducks taken at the same place during the same period consisted of 54% plant material with *Scirpus* seeds predominating, 31% *Chara* branches and oospores and 15% animal matter.

Forty-five coots taken at the same place during the same period had eaten *Chara* branches almost exclusively, the total percentage volume, including two small items of oospores, being 97%; the remainder consisted of other plant materials. The high percentage of *Chara* is taken to indicate a decided food preference; *Chara* oospores apparently are not sought for as would seem to be the case with ducks. *Scirpus* seeds, which occurred in small numbers in 25 stomachs, were in each case a minor item and probably taken incidentally to the *Chara*.

Cover and Food Plants

Bulrush—*Scirpus occidentalis*. Of the emergent plants the bulrush is the most important in the economy of the lake, providing cover and nesting material for many kinds of birds, as well as furnishing a seed crop of the highest value. Dry plants of the previous year's growth are used as nesting material by Redhead, Canvas-back, Ruddy duck, and coots; the thick clumps of dry growth, that have resisted winter storms and the weight of heavy snow, provide nesting cover; the rotted stems are often the chief constituent in the floating nests of grebe; the green stems represent the chief item in the diet of muskrats and are used in building their houses; many kinds of aquatic insect larvae use the stems as emerging ladders; molluscs cling to the under-surface portions of the plants; and finally the thick growth provides shelter for the propagation of lesser water plants and numerous small aquatic organisms.

The seeds are eaten by ducks, and to a lesser extent by coots, rails, waders, and other birds. The seeds ripen and begin to fall in September; many

TABLE II

AUTUMN FOOD OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA. NUMBER OF OCCURRENCES OF FOOD ITEMS

—	Number of specimens	*Chara* branches	*Chara* oospores	*Scirpus* seeds	*Potamogeton* seeds	Other seeds	Molluscs	Insects	Miscellaneous animals	Miscellaneous vegetation
Mallard	12	–	–	11	10	5	5	1	–	10
Gadwall	5	–	1	5	–	–	–	1	1	2
Baldpate	9	2	2	2	–	–	–	1	1	8
American Pintail	10	2	6	10	7	5	–	–	–	–
Green-winged Teal	10	–	7	9	3	2	1	4	–	2
Blue-winged Teal	2	–	1	1	–	1	2	1	–	–
Cinnamon Teal	5	–	1	5	5	2	4	5	–	2
Shoveller	6	–	2	4	5	2	6	–	1	1
Redhead	8	3	4	5	4	1	1	1	1	5
Ring-necked Duck	5	1	4	5	2	1	1	–	1	1
Canvas-back	1	1	–	–	1	1	–	–	–	–
Greater Scaup Duck	1	–	–	–	1	–	–	–	–	–
Buffle-head	6	–	–	6	5	–	2	4	4	2
Old-squaw	2	2	–	2	1	2	–	–	–	–
Ruddy Duck	9	–	5	8	1	1	–	1	1	2
American Coot	45	45	2	31	1	3	–	–	–	3

TABLE III

AUTUMN FOOD OF DUCKS AND COOTS, SWAN LAKE, BRITISH COLUMBIA, AVERAGE PERCENTAGE VOLUME

—	Number of specimens	*Chara* branches	*Chara* oospores	*Scirpus* seeds	*Potamogeton* seeds	Other seeds	Molluscs	Insects	Miscellaneous animals	Miscellaneous vegetation
Mallard	12	—	—	56.84	13.00	2.75	2.00	0.08	—	25.33
Gadwall	5	—	0.40	70.60	—	—	—	—	9.60	19.40
Baldpate	9	21.44	0.44	0.22	—	—	—	0.11	0.11	77.68
American Pintail	10	6.20	24.40	63.50	2.30	3.60	—	—	—	—
Green-winged Teal	10	—	24.30	39.80	5.70	6.00	0.60	10.10	—	13.50
Blue-winged Teal	2	—	0.50	45.00	—	2.50	14.50	37.50	—	—
Cinnamon Teal	5	—	—	65.20	4.80	0.60	25.20	3.40	—	0.80
Shoveller	6	—	0.50	24.83	50.00	0.83	23.00	—	0.17	0.67
Summary, pond ducks	59	4.32	8.40	44.82	9.48	2.38	5.45	3.28	0.85	21.02
Redhead	8	31.25	16.63	20.87	11.00	1.13	1.00	0.12	1.13	16.87
Ring-necked Duck	5	1.00	34.00	56.20	2.20	0.40	0.20	—	1.00	5.00
Canvas-back	1	50.00	—	—	40.00	10.00	—	—	—	—
Greater Scaup Duck	1	100.00	—	—	—	—	—	—	—	—
Buffle-head	6	—	—	25.60	19.13	—	15.14	13.13	22.50	4.50
Old-squaw	2	72.50	—	6.50	0.50	20.50	—	—	—	—
Ruddy Duck	9	—	16.70	52.67	—	0.22	—	17.75	0.11	12.55
Summary, diving ducks	32	17.15	14.15	34.28	7.92	1.96	3.11	7.48	4.65	9.30
American Coot	45	96.60	0.13	2.87	—	0.06	—	—	—	0.34

TABLE IV

FREQUENCY OF OCCURRENCE OF IDENTIFIED FOOD SUBSTANCES IN STOMACH CONTENTS OF 59 POND DUCKS, 32 DIVING DUCKS, AND 45 COOTS

Item	Pond ducks	Diving ducks	Coots
Musk-grass, *Chara* sp.	5	8	45
Chara oospores	21	13	2
Bulrush, *Scirpus occidentalis*, seeds	46	26	–
sp.	–	–	31
Pondweeds, *Potamogeton heterophyllus*, seeds	11	10	1
foliosus, seeds	2	1	–
pectinatus, seeds	26	6	–
pectinatus, rootlets	1	–	–
Horned pondweed, *Zannichellia palustris*, leaves	2	–	1
Knotweed, *Polygonum amphibium*, seeds	–	–	1
aviculare, seeds	3	–	–
muhlenbergii, seeds	2	–	–
Smartweed, *Polygonum hydropiper*, seeds	1	–	–
Black bindweed, *Polygonum convolvulus*, seeds	1	–	–
Filamentous algae, *Spirogyra*, *Zygnema*	3	2	–
Sedge, *Corex* sp., seeds	2	1	–
Grasses, Gramineae, leaves	5	–	–
Bromus sp., leaves	–	–	1
Water milfoil, *Myriophyllum spicatum*, seeds	6	2	–
Golden dock, *Rumex maritimus*, seeds	2	1	–
Hornwort, *Ceratophyllum demersum*, seeds	1	3	–
Bur-reed, *Sparganium* sp., seeds	–	1	–
Cat-tail, *Typha latifolia*, seeds	1	–	–
Nostoc, cells	1	–	–
Comminuted vegetable matter	10	6	–
Freshwater sponge, Porifera	1	1	–
Bristleworm, *Nais* sp.	1	–	–
Water boatmen, Corixidae	6	6	–
Midge, Chironomidae, adults	2	–	–
larvae	1	4	–
Beetle, Coleoptera, terrestrial	1	–	–
water beetle—Dytiscidae	1	–	–
Bryozoa, statoblasts	–	3	–
Waterflea, *Daphnia* sp.	–	1	–
Amphipods, *Gammarus limnaeus*	–	3	–
Sow-bug, Isopoda	–	1	–
May-fly, Ephemeroptera, nymph	–	1	–
Damsel-fly, Odonata, nymph	–	1	–
Dragon-fly, Odonata, nymph	–	1	–
Insects, unidentified fragments	5	2	–
Gastropods, *Planorbis* sp.	8	2	–
Physa gabbi	1	–	–
Physa sp.	2	–	–
Helisoma trivolvis	2	1	–
Molluscs, unidentified fragments	10	1	–
Fish, carrion	1	–	–

sink in the muddy shallows where they are available for food in the following spring. In short, the bulrush is considered to be the plant of chief importance in the life of the lake. It is perhaps unnecessary to add that any loss of this growth through burning, which sometimes happens, completely disrupts the economy of the burned area for some years.

Cat-tail—*Typha latifolia*. The remarks in connection with the value of the bulrush as a cover plant and its use in nest building are applicable also to the cat-tail. The latter, however, does not produce a seed of value to waterfowl and for this reason is of less importance.

Sedges—Carex sp. Various species of sedge occupy the outer edge of the marsh areas and surrounding wet lands beyond the cat-tail and bulrush zone. These produce seeds of value and provide cover for nesting pond ducks.

Pondweeds; other submerged plants. The sago pondweed, *Potamogeton pectinatus*, is the commonest of the pondweeds; next in importance is *P. heterophyllus*, and third is *P. pusillus*. Their distribution, and also that of Water milfoil, *Myriophyllum spicatum*, Bladderwort, *Utricularia* sp., and Hornwort, *Ceratophyllum demersum*, is limited and becomes dominant only in the shallows at the north and south ends of the lake, where sand is an important constituent of the lake bottom. In these places there is usually an association of several of the species mentioned, forming a thick mass of growth. Duckweeds, *Lemna minor*, *Lemna trisulca*, and *Spirodella polyrhiza*, occur in sheltered areas within the bulrush and cat-tail marshes. All these plants form part of the diet of ducks.

Musk-grass—*Chara* sp. This alga is dominant over most of the lake bottom. In summer, as the water level lowers, changes in the appearance of the visible growth in the shallow areas rapidly take place. What formerly were *Chara* meadows become sodden masses as the tall growth collapses with the falling water. As the lake continues to fall and the *Chara* in the shallows becomes exposed to the sun, the upper surface of the mass dries and crumbles. Meanwhile the growth in deeper portions, always brittle and deciduous, contributes its surplus to the accumulation, so that eventually long islands of the material are strung along the shore outside the line of rushes. These are used as resting places by pond ducks.

Chara forms the chief item in the food of coots, and is important in the diet of some diving ducks. It provides also suitable habitat for the propagation of insects, crustaceans, and other small animals which are eaten by waterfowl. *Chara* oospores, which accumulate about the beds in the shallows, are eaten by pond ducks and to a lesser extent by diving ducks.

Filamentous algae—*Spirogyra*, *Zygnema*. In early summer these hair-like algae accumulate in thick mats on the surface of the water and later are deposited in windrows along open spaces on the shore, or become attached to the marsh growth as the waters recede. This growth is important in the ecology of the lake; while in the water it provides cover for many kinds of small organisms, and when dry it is used as nesting material by grebe and other birds.

Conclusions

Observation of waterfowl at Swan lake, British Columbia, during a four-year period, and study of the stomach contents of 136 specimens, indicate

that competition for food between ducks and coots during the autumn months is negligible. The branches of *Chara* form the chief food of coots; the oospores of the alga and to a lesser extent the branches are eaten by some species of duck. As this is the most abundant growth in the lakes and is produced in unlimited quantities, there is sufficient for the requirements of a much greater coot and duck population than is ever likely to occur. It is probable that less than one per cent of the *Chara* crop is consumed by waterfowl. Seeds of *Scirpus* are most important in frequency of occurrence and percentage volume of duck food. Seeds of other aquatic plants and, to a lesser degree, molluscs and aquatic insects, also are eaten. These foods are inconspicuous items in the diet of coots.